Antiplatelet therapy and clinical outcomes in cardiovascular diseases

by

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Abstract

Cardiovascular diseases (CVD) is a leading cause of death in the world. Despite effective treatment regimens for ischaemic heart disease (IHD) and ischaemic stroke, mortality and recurrence rates remain high. Antiplatelet therapy is on effective treatment and reduces the risk of recurrent heart attack and stroke. Nevertheless, there are patients who stopped or interrupted their antiplatelet therapy for certain reasons or some patients may be resistant or poor responders to antiplatelet therapy. Furthermore, there is evidence of rebound effect in platelet activity after antiplatelet cessation and this may associate with increased risk of cardiovascular event. This thesis is divided into five main chapters (chapters 3 to 7) which attempt to provide data to help resolve the uncertainty.

Chapter 1 highlights the background of cardiovascular diseases and the global burden of cardiovascular and cerebrovascular diseases. The metabolism of platelets, antiplatelet therapy and current antiplatelet therapy guidelines are described, followed by discussion of the risk of cardiovascular event and changes in antiplatelet therapy.

Chapter 2 describes the data source from Virtual International Stroke Trial Archive (VISTA) and National Health Service Greater Glasgow and Clyde (NHSGGC) Safe Haven, followed by definition of outcome measures.

In chapter 3, Virtual International Stroke Trial Archive (VISTA) data was examined to test whether continue with the same antiplatelet therapy or changing to a new antiplatelet regimen reduces the risk of subsequent events in patients who experience a stroke whilst taking antiplatelet therapy. The findings indicate that subjects who switch to a new antiplatelet regimen after stroke did not have a lower early recurrence rate than subjects who continued with the same antiplatelet therapy. Observations on bleeding complications were similar in both groups. However, changing antiplatelet regimen after stroke was associated with more favourable functional outcome across a full scale modified Rankin Scale (mRS) at 90 days.

In chapter 4, association between early or later initiation of antiplatelet with a recurrent ischaemic stroke and bleeding complications was assessed using VISTA data. The findings indicate that there was no association between a recurrent ischaemic stroke and timing of initiation of antiplatelet drug after stroke. However, early initiation was associated with increased risk of bleeding. In terms of functional outcomes, this study demonstrated that the mid-time and late initiation of antiplatelet therapy after acute stroke are associated with better functional outcomes compared with early initiation.

In chapter 5, a nested case-control study was performed to explore the rate of antiplatelet cessation and interruption in a sample of patients with recent ischaemic stroke and to assess the risk of cardiovascular events associated with cessation and interruption of antiplatelet. It was found that there was no increased risk of cardiovascular event among patients who had early cessation or interrupted/stopped antiplatelet therapy within 90 days following acute ischaemic stroke.

In chapter 6, the incidence and predictors of cardiovascular events after DAPT cessation were evaluated. The incidence of cardiovascular event while taking DAPT and following discontinuation of DAPT was 15.7% and 16.7% respectively. This study found that increasing age was associated with an increased risk of cardiovascular event, whereas, revascularization-treated patients and longer duration of DAPT, were each associated with a decreased risk. The duration of DAPT six months and less was associated a significantly higher risk for cardiovascular event.

In chapter 7, an untargeted metabolomics analysis was performed while on DAPT (aspirin plus ticagrelor) and once they stopped ticagrelor to identify metabolite changes associated with cardiovascular events after stopping DAPT. Ten ACS patients were recruited in this study and data were analysed for seven patients. Three hundred eleven putative metabolites were identified. This study found 16 putative metabolites significantly altered following ticagrelor cessation. Of these, seven metabolites were from lipid pathway and down-regulated some up to 3-fold. On the other hand, adenosine, from nucleotide metabolism was upregulated up to 2.6-fold. It concluded that there are changes

in numerous pathways following DAPT discontinuation and whether these changes differ in patients who have cardiovascular event after stopping DAPT warrant further investigation.

In chapter 8, a summary of the findings of this thesis are presented as well as the future directions of research in this area.

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Papers

Mazlan-Kepli W, MacIsaac RL, Walters M, Bath PM, Dawson J. Antiplatelet therapy following ischaemic stroke – Continue or change pre-existing therapy? European Stroke Journal 2396987316678728, first published on November 4, 2016 as doi:10.1177/2396987316678728.

Conference proceedings

Chapter 3:

ISC 2014: (Oral presentation: Won a Travel Award for Junior Investigators and among top 10 percent of the accepted abstracts) Mazlan-Kepli W, Fulton RL, Walters M, Ali M, Bath PM, Dawson J, for the VISTA Collaborators. Abstract 135: Antiplatelet therapy following ischemic stroke - Continue or change pre-existing therapy? Stroke. 2014 February 1, 2014;45(Suppl 1):A135.

AHA Scientific Sessions (Best of AHA Speciality Conferences): (Poster presentation) Mazlan-Kepli W, Fulton RL, Walters M, Ali M, Bath PM, Dawson J, for the VISTA Collaborators. Abstract 10478: Antiplatelet therapy following ischemic stroke - Continue or change pre-existing therapy?

Chapter 4:

ESC 2014: (Poster presentation) Mazlan-Kepli W, Fulton RL, Walters M, Ali M, Bath PM, Dawson J, for the VISTA Collaborators. Abstract 1403: Very early initiation of antiplatelet therapy following ischaemic stroke is associated with lower recurrence rate. Cerebrovascular Diseases. 2014;37(Suppl 1):R1403.

Chapter 5:

15th Asian and Oceanian Congress of Neurology (AOCN 2016) (Poster presentation) Mazlan-Kepli W, Fulton RL, Walters M, Bath PM, Dawson J, for the VISTA Collaborators. Abstract 0070: Interruption to antiplatelet therapy early after acute ischaemic stroke: A nested case-control study.

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I am extremely grateful to Cardiac Rehabilitation Team from NHS Greater Glasgow and Clyde, Ms Gillian Armstrong, Ms Fiona Rigby, Ms Caroline Wilson and Ms Alison Shanks for their help with patient recruitment and without them it will be impossible for me to get in touch with acute coronary syndrome patients. I am grateful to Acute Stroke Unit Research Nurse, Ms Lesley McDonald and Ms Belinda Manak for their help and assistance with the prospective study. Their support has come from many different sources, to help with the blood sampling at the initial part of the study, they supervised my phlebotomy technique before I become competent in phlebotomy and collecting data from the NHS computer database.

I am very grateful to the Ministry of Health (MOH) Malaysia for the scholarship award and their generosity in providing funding to pursue my PhD. I would like to acknowledge the MOH Pharmaceutical Services Division and MOH Training Division for this opportunity. I would like to acknowledge my mentors in Malaysia for their friendship and support i.e. Mr Abdol Malek Abd Aziz and Ms. Saidatul Raihan Ibrahim.

Most importantly, I would like to thank the patients who agreed to participate in my prospective research project. I am quite surprised by the willingness to volunteer and consent to participation in this research. I am extremely grateful to these incredible people.

Finally, I would like to thank my husband, Mr Syahrul Ezanee Sulaiman, sacrificed many things and supported me all the way throughout this PhD journey. Without him, I will not be here. To my parents and family in-laws, love and thanks to them for believing in me. Thanks also to all those whom I met over the last years, especially Ms Sukainah Al-Shaikh who has been there listening as my friends.

Author's declaration

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original and my own work, unless specified otherwise in the text. Data from the Virtual International Stroke Trial Archive (VISTA) were extracted with help from Dr Jesse Dawson and Dr Rachael MacIsaac. The use of the anonymised database for analyses is approved by the VISTA Steering Committee. The Safe Haven database developed by NHS Greater Glasgow and Clyde (NHS GG&C) are extracted with help from Dr Jesse Dawson and Ms Claire MacDonald. The use of the database for analyses is approved by the Safe Haven team (GSH/13/CA/005). The prospective study has been approved by the NRES Committee North West - Greater Manchester East (14/NW/1163).

This work has never previously been submitted for a higher degree. I conducted all research at the Institute of Cardiovascular and Medical Sciences, University of Glasgow and always under the supervision of Dr Jesse Dawson and Prof Matthew Walters.

Wardati Mazlan-Kepli
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List of abbreviations, acronyms & symbols

5HT	Serotonin
5HT2A	Serotonin 2A receptor,
11-dh-TXB_2	11-dehydro-thromboxane B2
αIIbβ3	Glycoprotein IIb/IIIa
AA	Arachidonic acid
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
AE	Adverse event
AF	Atrial fibrillation
AMI	Acute myocardial infarction
AP	Antiplatelet
ARB	Angiotensin receptor blocker
ATC	Anatomical Therapeutic Chemical
ATP	Adenosine triphosphate
BMS	Bare metal stent
BNF	British National Formulary
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence interval
CK	Creatinine kinase
CNS	Central nervous system
COX	Cyclooxygenase
CRF	Case report form
CRP	C-reactive protein
CV	Cardiovascular event
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DALY	Disability-adjusted life year
DAPT	Dual antiplatelet therapy
DES	Drug eluting stent
ECG	Electrocardiographic
ECH	Extracerebral haemorrhage
ERDP	Extended-release dipyridamole
ESO	European Stroke Organisation
FDA	Food and drug administration
GI	Gastrointestinal
GP	Glycoprotein

GRACE	Global Registry of Acute Coronary Events
HR	Hazard ratio
ICD-10	International Classification of Diseases, Tenth Revision
ICH	Intracranial haemorrhage
IHD	Ischaemic heart disease
IL	Interleukin
IQR	Interquartile range
IRR	Incidence rate ratio
ISD	Information services division
LC-MS	Liquid Chromatography Mass Spectrometry Analysis
LOQ	Limit of quantitation
LV	Left ventricular
LVEF	Left ventricular ejection fraction
MACE	Major adverse cardiovascular events
MI	Myocardial infarction
MMAS-4	Morisky medication adherence scales
mRS	Modified Rankin Scale
NICE	National Institute for Health and Care Excellence
NIHSS	National Institutes of Health Stroke Scale
NHS	National Health Service
NSAIDS	Nonsteroidal anti-inflammatory drugs
NSTEMI	Non ST elevation myocardial infarction
OPCS-4	Office of Population Censuses and Surveys
OR	Odds ratio
PAD	Peripheral arterial disease
PAI-1	Plasminogen activator inhibitor
PAR	Protease-activated receptor
PCI	Percutaneous coronary intervention
PDE	Phosphodiesterase
PE	Pulmonary embolism
PTCA	Percutaneous transluminal coronary angioplasty
QC	Quality control
RR	Risk ratio
RRR	Relative risk reduction
rt-PA	recombinant tissue plasminogen activator
SAE	Serious adverse event
sCD40L	Soluble CD40-ligand
SCI-DC	Scottish Care Information – Diabetes Collaboration
SD	Standard deviation
SIGN	Scottish Intercollegiate Guidelines Network
SMC	Smooth muscle cell

SMR01	Scottish Morbidity Records
STEMI	ST elevation myocardial infarction
TIA	Transient ischaemic attack
TIMI	Thrombolysis in Myocardial Infarction
tPA	Tissue plasminogen activator
TPa	Thromboxane receptor protein antagonist
TX A_2	Thromboxane A2
uPA	Urokinase-type plasminogen activator
USA	Unstable angina
VISTA	Virtual International Stroke Trials Archive
VKA	Vitamin K antagonist
vWF	von Willebrand factor
WHO	World Health Organisation

1 Introduction

1.1 Antiplatelet therapy and cardiovascular diseases

Antiplatelet therapy is a key in pharmacological treatment for prevention of coronary heart disease (CHD) and stroke. Depending on indication, duration of antiplatelet monotherapy or dual therapy is varied. Antiplatelet therapy is indicated to prevent a recurrence of cardiovascular event, however, extended duration of dual antiplatelet therapy (DAPT) associated with increased risk of bleeding. Furthermore, antiplatelet therapy itself is known to associate with adverse events (AE) like gastrointestinal and non-gastrointestinal bleeding, intracranial haemorrhage (ICH) and allergic reactions. Thus, this leads to premature discontinuation of antiplatelet therapy which later associates with increased risk of cardiovascular event after antiplatelet cessation. In this chapter, I am going to discuss the background of cardiovascular diseases and antiplatelet therapy used in patients with cardiovascular disease.

1.2 Cardiovascular diseases

Cardiovascular disease (CVD) is defined by World Health Organisation (WHO) as any disorder related to the heart and blood vessels. It is the most serious and common disease in the world. CVD disorders include CHD, stroke (cerebrovascular disease), hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. In 2015, CVD was the leading cause of death from non-communicable diseases (GBD 2015 Mortality and Causes of Death Collaborators, 2016). It is estimated that approximately 17.9 million people died due to CVD in 2015. The number of global deaths due to cardiovascular disease increased by 12.5% between 2005 and2015 (GBD 2015 Mortality and Causes of Death Collaborators, 2016). Circulatory disease and CVD accounts for 11.8% of global DALY (Murray, et al., 2012).

In this section, I am going to discuss in depth CHD and stroke exploring the burden of these two disorders, their pathophysiology, signs and symptoms and evidence based treatment.

1.3 Coronary heart disease

1.3.1 Background

Coronary heart disease is also known as ischaemic heart disease (IHD) and can present with symptoms of stable angina, sudden death, an acute coronary syndrome (ACS) or symptoms of heart failure. ACS are caused by disruption of blood flow to the heart muscle due to rupture of fatty substance or atherosclerotic plaques in coronary arteries (Grech and Ramsdale, 2003). Traditionally, ACS is subdivided into unstable angina, non-ST elevation myocardial infarction (NSTEMI) and ST elevation myocardial infarction (STEMI). Unstable angina occurs due to partially or totally block of the blood coronary blood vessel leading to myocardial ischaemia. Acute myocardial infarction occurs due to prolonged myocardial ischaemia which causes myocardial necrosis (Thygesen, et al., 2012).

Coronary heart disease is the leading cause of death globally (GBD 2015 Mortality and Causes of Death Collaborators, 2016). In 2015, CHD killed 8.9 million people, and deaths increased by 16.6% between 2005 and 2015. Its has an impact worldwide, in every country and population. CHD burden globally was 150 million DALY in 2013, which represents an increase of 8.4% compared to 2005 (GBD 2013 DALYs and HALE Collaborators, 2015). In the United Kingdom (UK), CHD burden was 1.4 million in 2010 (Murray, et al., 2013). In Scotland, it is estimated 8000 people die from CHD and in 2012, it is estimated around 7.3% male and 5.7% female are living with CHD (NHS National Services Scotland, 2014).

One month after the acute event, patients with ACS are very vulnerable physically, mentally and emotionally due to chest pain and anxiety (Kristofferzon, et al., 2005; Bach, et al., 2011; Rančić, et al., 2013). Besides that, the rates of all-cause mortality increased in the first 6 months, especially in the first 30 days after an acute episode of ACS (Das, et al., 2006). In Scotland, around 92% of people survived 30 days or more following heart attack in year 2012/2013 and the age and sex standardised mortality rate for heart attacks was 47.4 per 100,000 population in the same year (NHS National Services

Scotland, 2014). In terms of risk of recurrence AMI, a study found about 14% men and 17% women who survived their first AMI experienced a recurrence AMI within 7 years (Smolina, et al., 2012).

In order to minimise risk of recurrence and death, patients with ACS are typically offered pharmacotherapy in the form of antiplatelet therapy, lipid lowering therapies and blood pressure lowering drugs and many undergo procedures such as percutaneous angioplasty and stenting. Dual antiplatelet therapy (DAPT) is normally prescribed following ACS. At present there are few combinations of DAPT available such as aspirin plus clopidogrel or aspirin plus ticagrelor. Current guidelines recommend that DAPT should be continued for between three and 12 months after the acute episode (O'Gara, et al., 2012; Scottish Intercollegiate Guidelines Network (SIGN), 2013a). After this period, all patients with ACS will continue aspirin in whom it is not contraindicated for lifelong to prevent a recurrence of ACS.

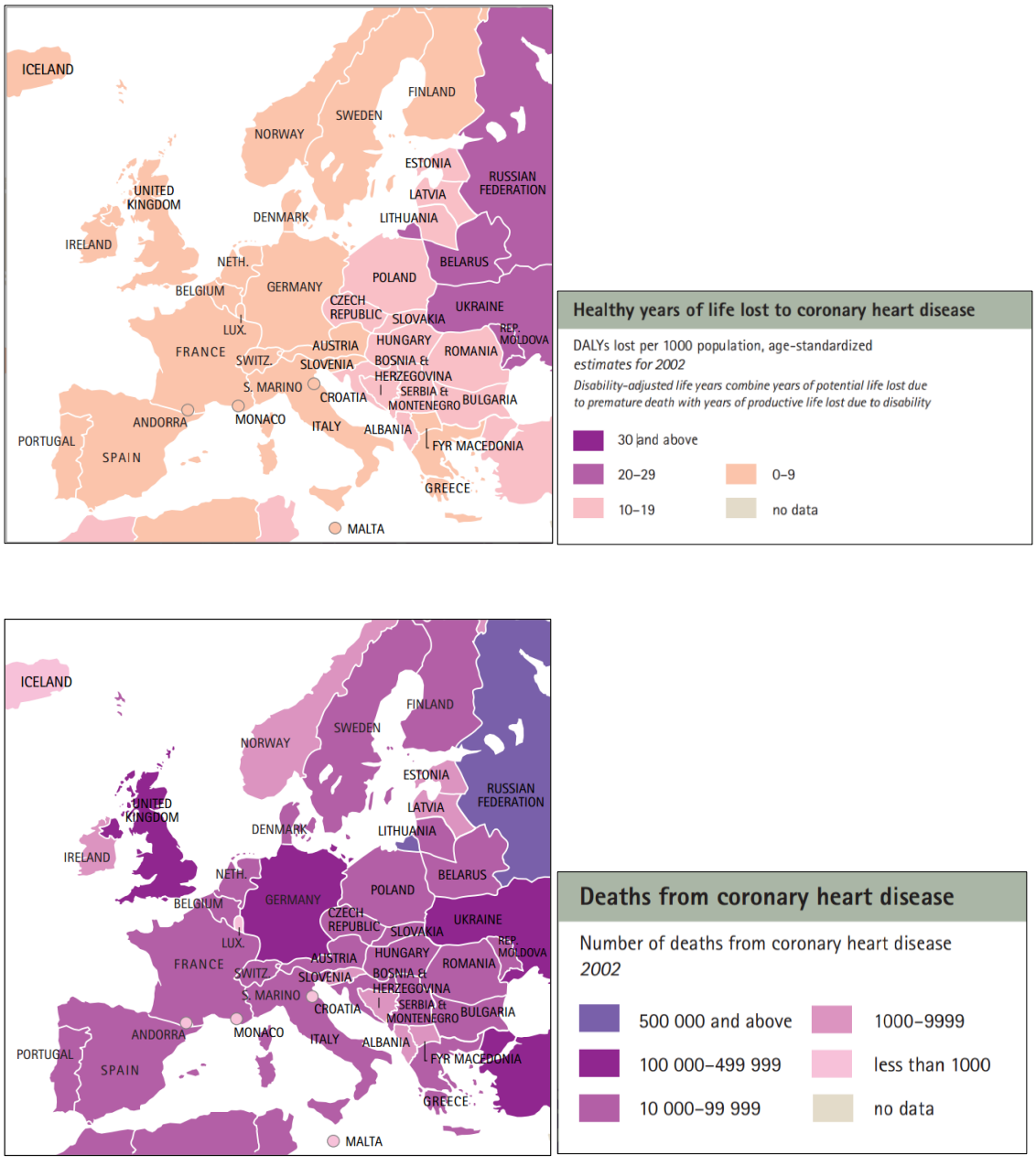


Figure 1-1: Healthy years of life lost (above) and mortality rates (below) attributed to coronary heart disease within European countries.

Source: www.who.int (Permissions obtained from World Health Organization (Permission authorization for WHO copyrighted material); ID number 196107 & 196108) (World Health Organization, 2004c; World Health Organization, 2004a)

1.3.2 Pathophysiology of acute coronary syndrome

Acute coronary syndrome is caused by atherosclerotic plaque build-up inside coronary arteries. The atherosclerotic plaque narrows the coronary arteries and decreases blood flow to the heart. There are two forms of atherosclerotic lesions in human coronary arteries; stenotic and non-stenotic lesions (Libby and Theroux, 2005). Stenotic lesions are few but in the form of smaller lipid cores and covered with thick fibrous caps. Non-stenotic lesions tend to have large lipid cores with thin fibrous caps and are vulnerable to rupture and thrombosis.

The fibrous caps of atherosclerotic plaque consists of macrophages and smooth muscle cells and may disintegrate, tear or break at any point (Falk, et al., 1995) This will cause the atherosclerotic plaque to be exposed to the flowing blood and provoke thrombosis. Platelet activation is triggered by the collagen (produced by the smooth muscle cells in the plaque) and the coagulation cascade is activated by macrophages and smooth muscle cells (Libby and Theroux, 2005). Once platelets become activated, fibrin is formed and von Willebrand factor is released (Figure 1-2). The thrombus can either grows until it occludes the blood vessel or breaks away from the atherosclerotic plaque and embolise distally (Guyton and Hall, 2006).

The resting coronary blood flow in the heart is about 225 ml/min, which is 70 ml/min/100g of cardiac tissue (Guyton and Hall, 2006). When the occlusion of coronary arteries more than 80%, this condition will cause ischaemia to the surrounding tissue due to the insufficient blood flow to cardiac muscle and lack of oxygen. Extreme cardiac ischaemia will lead to infarction to the tissue (Guyton and Hall, 2006). For unstable angina and NSTEMI, coronary arteries are partially or intermittently occluded, whereas in STEMI, there is typically complete occlusion (Kumar and Cannon, 2009; Overbaugh, 2009).

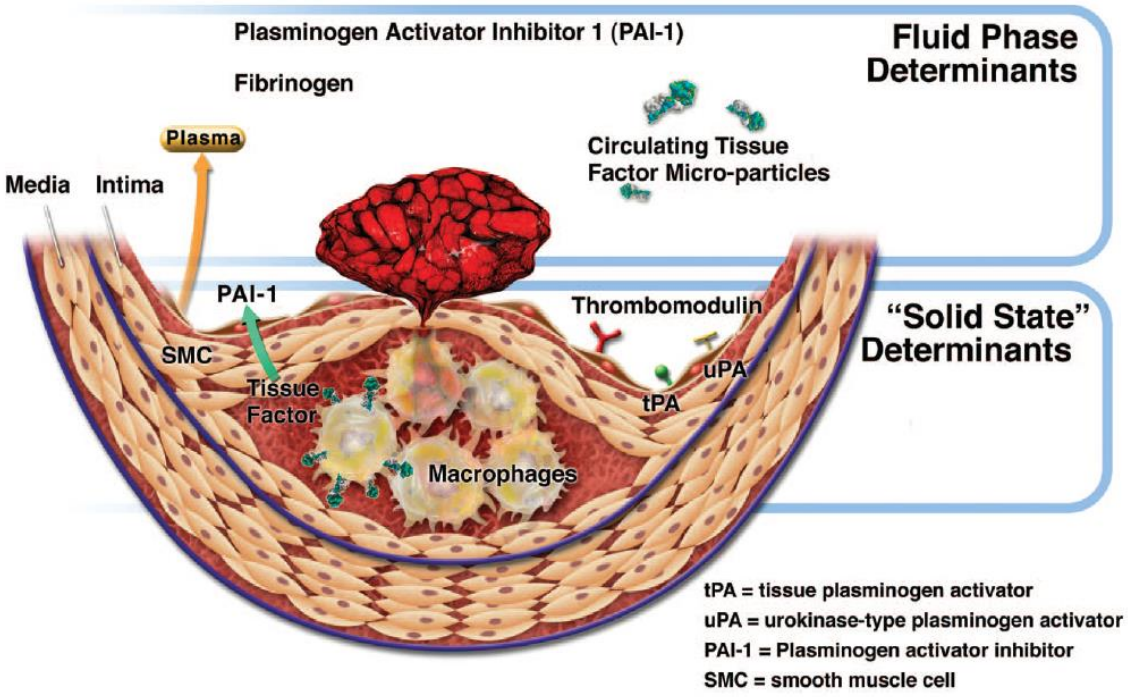


Figure 1-2: Determinants of thrombosis in coronary atherosclerotic plaques.

tPA=tissue plasminogen activator, uPA=urokinase-type plasminogen activator, PAI-1=plasminogen activator inhibitor, SMC=smooth muscle cell. Adapted from Libby and Theroux (2005). (Permissions obtained from Wolters Kluwer Health, License Number 3785951496889)

1.3.3 Differences between types of acute coronary syndrome

There are three types of ACS that occur due to an atherosclerotic plaque rupture and the abrupt narrowing and occlusion in the coronary arteries. The type of ACS is depending on the location of the blockage, the amount of time that blood flow is blocked and the amount of damage to the myocardium area. Diagnosis of ACS are depending on the clinical presentation, electrocardiographic (ECG) changes and biochemical cardiac markers (Scottish Intercollegiate Guidelines Network (SIGN), 2013a).

Unstable angina does not cause myocardial damage, but it may progress to myocardial infarction. Signs and symptoms include pain with or without radiation to the arm, neck or epigastric region and shortness of breath, diaphoresis, and nausea. Signs and symptoms of unstable angina are less severe

than in ST- and NSTEMI and the diagnosis is supported by the absence of ECG changes and with normal cardiac biomarkers (Overbaugh, 2009).

NSTEMI is less serious than STEMI as the blood supply to the myocardium is not completely blocked but it still causes damage to the myocardium. The myocardial damage is followed by the release of a biomarker of myocardial necrosis into the circulation, i.e. troponins T or I, or creatinine kinase (CK-MB) (Kumar and Cannon, 2009). Signs and symptoms of NSTEMI can be more severe than in unstable angina. Cardiac biomarkers are also elevated with ECG changes show either ST-segment depression or T-wave inversion.

STEMI is the most serious types of ACS. A thrombus fully occludes the coronary artery and caused infarction and ischaemia to the surrounding tissue of myocardium. Signs and symptoms of STEMI are longer in duration and more severe than in unstable angina. Cardiac biomarkers are elevated in this type of ACS with ECG changes show ST-segment elevation or a new left bundle branch block (Overbaugh, 2009).

1.3.4 Current management of acute coronary syndrome

Once a patient has arrived at the hospital and received a diagnosis of ACS, management aims to decrease the extent of myocardial damage and prevent major cardiovascular events such as death and nonfatal MI. The therapeutic approach for ACS are depending on the underlying pathophysiology of unstable angina, NSTEMI and STEMI.

In unstable angina and NSTEMI, the related coronary artery is usually partially or intermittently occluded, thus the goal of treatment is to prevent further thrombosis, reocclusion and recurrent ischaemia. In early management, DAPT and anticoagulant should be offered. For intermediate and high risk patients (based on Global Registry of Acute Coronary Events [GRACE] or Thrombolysis in Myocardial Infarction [TIMI] scoring system), coronary angiography will be offered and follow with percutaneous coronary intervention (PCI) if indicated (National Institute for Health and Care Excellence, 2010; Scottish Intercollegiate Guidelines Network (SIGN), 2013a). Once a patient has

stabilized, lipid-lowering therapies, beta-blocker and angiotensin-converting-enzyme (ACE) inhibitor are used over the long term. The medications and doses of therapy used for ACS are shown in Table 1-1.

Whereas in STEMI, as the coronary artery is totally occluded, the goal is to obtain normal coronary blood flow via pharmacological approach or catheter-based reperfusion. Percutaneous coronary intervention should be initiated within 120 minutes of ECG diagnosis or if this cannot be provided, a patient should receive thrombolytic therapy such as streptokinase (O'Gara, et al., 2012; Scottish Intercollegiate Guidelines Network (SIGN), 2013a). The timing of reperfusion therapy in STEMI treatment is important to improve patient outcomes (Andersen, et al., 2003a; Andersen, et al., 2003b). The rest of pharmacological treatment should be initiated as soon as possible or once a patient has stabilized (Table 1-1).

Table 1-1: Selected routine medical therapies

Therapy	Medications
Unstable angina / NSTEMI	
Antiplatelet	Aspirin 300mg stat, then 75mg daily Clopidogrel 600mg stat, then 75mg daily Prasugrel 60mg stat, then 10mg daily Ticagrelor 180mg stat, then 90mg twice a day
Anticoagulant	Unfractionated heparin iv bolus 60ii/kg then infusion 12ii/kg/h for 48 hours Enoxaparin 1mg/kg sc every 12 hours up to 8 days Fondaparinux 2.5mg iv, then 2.5mg sc daily up to 8 days
Anti-ischaemic	Nitroglycerin 0.4 mg sublingual every 5 min
Beta-blocker	Metoprolol 25-50mg every 6-12 hours Carvedilol 6.25-25mg twice daily
ACE inhibitor / ARB	Lisinopril 2.5-10mg daily Captopril 6.25-50mg three times daily Ramipril 2.5-5mg twice daily Valsartan 20-160mg twice daily
Cholesterol-lowering	Atorvastatin 80mg daily
STEMI	
Fibrinolytic	Tenecteplase Single iv weight-based bolus Reteplase 10ii + 10ii iv bolus given 30 min apart Alteplase 90-min weight-based infusion Streptokinase 1.5 MU iv over 30-60min
Antiplatelet	Aspirin 300mg stat, then 75mg daily Clopidogrel 600mg stat, then 75mg daily Prasugrel 60mg stat, then 10mg daily Ticagrelor 180mg stat, then 90mg twice a day
Anticoagulant	Unfractionated heparin iv bolus 60ii/kg then infusion 12ii/kg/h for 48 hours Enoxaparin 1mg/kg sc every 12 hours up to 8 days Fondaparinux 2.5mg iv, then 2.5mg sc daily up to 8 days
Anti-ischaemic	Nitroglycerin 0.4 mg sublingual every 5 min
Beta-blocker	Metoprolol 25-50mg every 6-12 hours Carvedilol 6.25-25mg twice daily
ACE inhibitor / ARB	Lisinopril 2.5-10mg daily Captopril 6.25-50mg three times daily Ramipril 2.5-5mg twice daily Valsartan 20-160mg twice daily
Cholesterol-lowering	Atorvastatin 80mg daily

ACE=angiotensin-converting-enzyme, ARB=Angiotensin receptor blocker, mg=milligrams, MU=mega unit, iv=intravenous, sc=subcutaneous.
(O'Gara, et al., 2012; Scottish Intercollegiate Guidelines Network (SIGN), 2013a)

1.4 Stroke

1.4.1 Background

Forty years ago, the WHO introduced definition of stroke as *'rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin'* (Aho, et al., 1980). In 2013, the American Heart Association/American Stroke Association suggested a new definition of stroke reflecting the greater understanding of pathophysiology and the wide spectrum of cerebrovascular diseases (Table 1-2). There are two major subtypes of stroke; ischaemic stroke and haemorrhagic stroke. Ischaemic stroke occurs due to reduction blood flow to the brain and causing neurological dysfunction and infarction. Haemorrhagic stroke occurs after rupture of a blood vessel in brain tissue.

In 2015, 6.3 million people died of cerebrovascular disease. Of these, about 3 million people died of ischaemic stroke and 3.3 million died of haemorrhagic stroke (GBD 2015 Mortality and Causes of Death Collaborators, 2016). In 2005, stroke burden was 107 million DALYs and the number increased to 112 million DALYs in year 2015 (GBD 2013 DALYs and HALE Collaborators, 2015). In 2010, stroke was the third leading cause of years of life lost in the UK (Murray, et al., 2013). Many stroke survivors suffer prolonged disability and neurological complications such as cognitive decline, dementia, depression or seizures (O'Brien, et al., 2003; Rothwell, et al., 2004; Hong, et al., 2010). The UK spends about 9 billion pounds a year caring for these patients (Saka, et al., 2009).

A patient who presents with an acute onset of ischaemic stroke may be offered a therapy with fibrinolytic treatment within 4.5 hours and oral aspirin antiplatelet therapy within 24 to 48 hours after stroke onset. A patient with a first-ever ischaemic stroke, single antiplatelet therapy is offered normally aspirin unless contraindicated. As per guidelines, this treatment should be continued for a life-long.

Table 1-2: Definition of stroke

The term “stroke” should be broadly used to include all of the following:
Definition of CNS infarction: CNS infarction is brain, spinal cord, or retinal cell death attributable to ischemia, based on 1. pathological, imaging, or other objective evidence of cerebral, spinal cord, or retinal focal ischemic injury in a defined vascular distribution; or 2. clinical evidence of cerebral, spinal cord, or retinal focal ischemic injury based on symptoms persisting ≥24 hours or until death, and other etiologies excluded. (Note: CNS infarction includes hemorrhagic infarctions, types I and II; see “Hemorrhagic Infarction.”) **Definition of ischemic stroke:** An episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction. (Note: Evidence of CNS infarction is defined above.) **Definition of silent CNS infarction:** Imaging or neuropathological evidence of CNS infarction, without a history of acute neurological dysfunction attributable to the lesion. **Definition of intracerebral hemorrhage:** A focal collection of blood within the brain parenchyma or ventricular system that is not caused by trauma. (Note: Intracerebral hemorrhage includes parenchymal hemorrhages after CNS infarction, types I and II—see “Hemorrhagic Infarction.”) **Definition of stroke caused by intracerebral hemorrhage:** Rapidly developing clinical signs of neurological dysfunction attributable to a focal collection of blood within the brain parenchyma or ventricular system that is not caused by trauma. **Definition of silent cerebral hemorrhage:** A focal collection of chronic blood products within the brain parenchyma, subarachnoid space, or ventricular system on neuroimaging or neuropathological examination that is not caused by trauma and without a history of acute neurological dysfunction attributable to the lesion. **Definition of subarachnoid hemorrhage:** Bleeding into the subarachnoid space (the space between the arachnoid membrane and the pia mater of the brain or spinal cord). **Definition of stroke caused by subarachnoid hemorrhage:** Rapidly developing signs of neurological dysfunction and/or headache because of bleeding into the subarachnoid space (the space between the arachnoid membrane and the pia mater of the brain or spinal cord), which is not caused by trauma. **Definition of stroke caused by cerebral venous thrombosi**s: Infarction or hemorrhage in the brain, spinal cord, or retina because of thrombosis of a cerebral venous structure. Symptoms or signs caused by reversible edema without infarction or hemorrhage do not qualify as stroke. **Definition of stroke, not otherwise specified:** An episode of acute neurological dysfunction presumed to be caused by ischemia or hemorrhage, persisting ≥24 hours or until death, but without sufficient evidence to be classified as one of the above.

Ref: Sacco, et al. (2013)

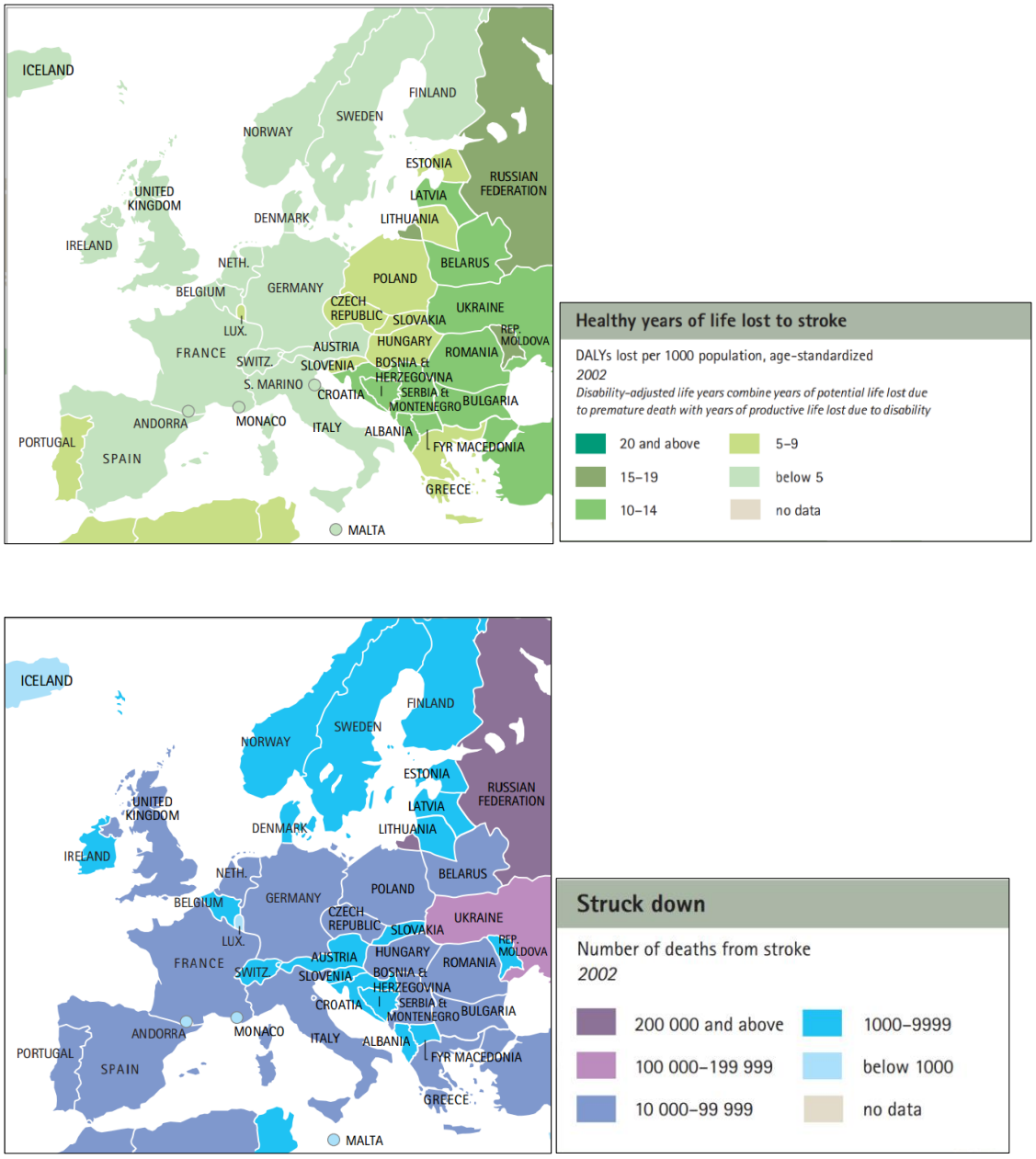


Figure 1-3: Healthy years of life lost (above) and mortality rates (below) attributed to stroke within European countries.

Source: www.who.int (Permissions obtained from World Health Organization (Permission authorization for WHO copyrighted material); ID number 196109 & 196110). (World Health Organization, 2004d; World Health Organization, 2004b)

1.4.2 Pathophysiology of ischaemic stroke

Ischaemic stroke occurs due to occlusion of intracranial arteries the blood vessels that supply blood to the brain tissue. The occlusion can be caused by a thrombus formed once the atherosclerotic plaque ruptures and activates platelet aggregation. The occlusion can also be caused by embolus, blood clot or other particles, travelling in the arteries from the central circulation. This obstruction leads to a reduction of blood flow in the brain that will cause lack of oxygen, brain ischaemia and acute loss of brain function in a localized area.

The normal rate of blood flow through the brain is between 50-65ml/min/100g of brain tissue. Neuron, a nerve cell in the brain requires a constant supply of oxygen and glucose and susceptible to hypoxic changes. Once blood flow reduces below 15ml/min/100g, ischaemic cascade at cellular level stimulates (Paciaroni, et al., 2009). Ischaemic cascade causes loss of potassium ion and adenosine triphosphate (ATP), thus lead to malfunction of an ion gradient. At the same time, ischaemia releases glutamate and aspartate, excitatory neurotransmitters in the brain, which leads to activation of disordered enzymes (e.g. lipases, proteases) and ultimately leads to cell death (Deb, et al., 2010). The extent of brain tissue damage is depending on the severity of ischaemia. If tissue ischaemia is mild, restoring blood flow may reduce and reverse the brain injury as the area is not yet infarcted (penumbra). However, if severe ischaemia happens the affected tissues dies (infarction) (Giraldo, 2013).

1.4.3 Types of ischaemic stroke

The complete occlusion of an artery may lead to an ischemic infarction, an area of necrotic cells caused by the obstruction of blood flow. There are four main types of ischaemic stroke i.e. cardioembolic, cryptogenic, small vessel and large artery disease. Large vessel disease is caused by ischaemia via embolism or reduction of blood flow. Whereas, small vessel disease is caused by accumulation of hyaline (a pathological protein) in the subendothelial which leads to narrowing and occlusion of a single deep perforating artery (Brainin and Heiss, 2009). Narrowing of carotid arteries in the neck and small arteries inside the brain, usually because of atherosclerotic plaque, reduces blood flow

to the brain. The complete occlusion of an artery may lead to an ischaemic infarction. Around 20% of ischaemic strokes are due to occlusion of carotid artery (Petty, et al., 1999). On the other hand, the occlusion of arteries caused by an embolus is called embolic stroke. The embolus carried in the bloodstream will lodge in the intracranial artery and caused occlusion which will lead to a reduction of blood flow (Frizzell, 2005; Deb, et al., 2010).

Diagnosis of stroke is important to provide optimal patient care and treatment. Diagnosis of stroke should include a full neurological and cardiovascular assessment; diagnostic testing (e.g. ECG, chest x-ray, blood work) and brain imaging (computed tomographic scan or magnetic resonance imaging) (Frizzell, 2005). A graded stroke severity assessment, the National Institute of Health stroke scale (NIHSS), would be used to rate the severity as a result of stroke (National Institute of Neurological Disorders and Stroke, 2008). Signs and symptoms of ischaemic stroke are depending on the area of the brain affected (Table 1-3).

1.4.4 Current management of stroke

Once a patient has arrived at the hospital and diagnosis has been made, the aims of management are to preserve tissue in the ischaemic penumbra, reduce the high risk of recurrent stroke and disability after stroke. The therapeutic approach for acute ischaemic stroke is to limit the severity of ischaemic tissue.

In early management of ischaemic stroke, the time window after stroke onset is important as this is the fibrinolytic therapy (alteplase) is administered. Alteplase is recommended for patients, who met the eligibility criteria, be treated within 4.5 hours after the onset of ischaemic stroke (Hacke, et al., 2008a; Scottish Intercollegiate Guidelines Network (SIGN), 2008; Wahlgren, et al., 2008; Kernan, et al., 2014). Aspirin, should be initiated within 24 to 48 hours after stroke onset, but it should be delayed for 24 hours if patients received alteplase.

Table 1-3: Clinical presentation in ischaemic stroke (Brainin and Heiss, 2009)

Artery	Symptoms
Anterior cerebral (Arboix, et al., 2009; Brainin and Heiss, 2009)	dysarthria, aphasia; unilateral contralateral motor weakness (leg/shoulder > arm/hand/face); minimal sensory changes (two-point discrimination) in the same distribution as above left limb apraxia; urinary incontinence
Middle cerebral	contralateral hemiparesis, hemi-hypesthesia, hemianopsia, and ipsilateral conjugated eye and head deviation; left lesion: aphasia and ideomotor apraxia; right lesion: contralateral multimodal hemineglect (visual, motor, sensitive, visual, spatial, auditive), anosognosia (denial of illness), anosodiaphoria (indifference to illness)
Vertebral	contralateral hemiparesis sparing the face (corticospinal tract), contralateral lemniscal sensory loss (medial lemniscus) and ipsilateral tongue paresis; contralateral thermoalgesic deficit (spinothalamic tract), dysphagia, dysphonia, pharynx and vocal cord weakness
Basilar	paresthesias, dysarthria, hemiparesis, dizziness
Posterior cerebral	dyschromatopsia, visual agnosia or alexia-without agraphia with a left lesion, spatial disorientation (topographagnosia), palinopsia, amusia, Balint syndrome (asimultanognosia or incapacity to see a scene as a whole, ocular apraxia or poor hand–eye coordination and optic ataxia or apraxia of gaze), metamorphosia, and prosopagnosia

For a patient with embolic stroke from cardiac source should be offered vitamin K antagonist (VKA) i.e. warfarin (Kernan, et al., 2014). There is likelihood that cardiogenic embolism happened in patients with atrial fibrillation; acute myocardial infarction and left ventricular thrombus; cardiomyopathy; vulvular heart disease and prosthetic heart valves. Other than warfarin, newer agent such as apixaban, dabigatran and rivaroxaban are all indicated in patients with nonvulvular atrial fibrillation to prevent future stroke

On the other hand, antiplatelet therapy is recommended for patients with non-cardioembolic stroke (Kernan, et al., 2014). Initiation of aspirin or clopidogrel as a monotherapy or combination of aspirin and extended-release dipyridamole is indicated after ischaemic stroke. However, in a patient who had an ischaemic stroke while taking aspirin, currently, there is no study to show that single agent or combination treatment is appropriate for this patient. Secondary preventative medications should be initiated as soon as possible and these may include antihypertensive, lipid-lowering therapies and lifestyle modification.

1.5 Platelet metabolism

Platelets, the smallest of human blood cells, were first described in 1865 by Professor Max Schultze (1825-1874). The discovery of the role of platelets in haemostasis was later established by Dr Giulio Bizzozero (1846-1901) (Brewer, 2006). Platelets are produced in the bone marrow and released in the form of megakaryocytes into the blood circulation (Thon and Italiano, 2010). A mature platelet with a diameter size 2-3micrometer is usually remains alive for about 5 to 9 days. Only 2/3 of the platelets circulate in the blood and the rest is stored in the spleen. The normal platelet count in the body is about 150,000-400,000 per microliter of blood. In a day, a healthy adult can produce about 1,000 million platelets and for old platelets, they are destroyed in the spleen and liver (Ghoshal and Bhattacharyya, 2014).

Platelets are essential in haemostasis. In normal condition, when the endothelium remains biochemically and physically intact, platelets remain in an inactive state. When there is vascular injury, collagen and von Willebrand factor (vWF) in the endothelium wall are exposed. Platelets in the blood circulation bind to the exposed collagen through interaction with glycoprotein VI/Ia receptor on the platelet surface and the exposed vWF binds to glycoprotein Ib/V/IX receptor. These bindings form a monolayer of activated platelets and lead to platelet adhesion (Brass, 2003; Jennings, 2009). Then, the adherent platelets release adenosine diphosphate (ADP) and thromboxane A2 (TxA2) where it leads to platelets change in shape and platelet activation.

When platelets become activated, platelet activation is initiated via multiple pathways. Other than stimulated by collagen exposure, ADP and TxA2, serotonin and thrombin pathways are also involving in platelet activation (Brass, 2003; Davi and Patrono, 2007; Varga-Szabo, et al., 2008). Secretion and accumulation of these agonists leads to recruitments of platelets from the blood circulation and formed platelet aggregations or stable platelet plugs. This stage is also known as primary aggregation. The secretion of platelet activators such as ADP, TxA2 and other substances, initiate secondary aggregation by provide positive feedback and strengthen the platelet-rich clot (Ghoshal and

Bhattacharyya, 2014). These processes lead to wound healing of the vascular injury.

Platelets are also responsible for the formation of arterial thrombus that normally occurs at sites of atherosclerosis and results in acute coronary syndrome or cerebral infarction (Ghoshal and Bhattacharyya, 2014). Rupture of atherosclerotic plaque will expose collagen and tissue factor to circulating platelets and thus promoting platelet activation and coagulation cascade. Activated platelets stimulate production of thrombin from prothrombin. Then, thrombin converts fibrinogen to fibrin leading to the formation of platelet and fibrin plug and further platelet activation and aggregation (Libby and Theroux, 2005; Guyton and Hall, 2006). This process can lead to thrombus formation, ischaemia and infarction of the affected area.

1.5.1 Adenosine diphosphate

ADP released from damaged endothelium and also from activated platelets will bind to platelet ADP receptors and consequently releasing more ADP (Geiger, et al., 1999; Kunapuli, et al., 2003). This will lead to further enhance platelet activation and recruit more platelets by coupled to inhibitory G-protein and release of intracellular calcium. The available ADP receptor antagonists are thienopyridines i.e. clopidogrel, ticlopidine and prasugrel; and non-thienopyridine i.e. ticagrelor. Mechanism of actions of these antiplatelet agents have been discussed in section 1.4.2. There are two ADP receptors on the platelet membrane i.e. $P2Y_1$ and $P2Y_{12}$. When the antiplatelet binds to the $P2Y_1$ receptor, it will result in release of intracellular calcium and leads to transient inhibition of platelet aggregation. On the other hand, binding to the $P2Y_{12}$ receptor results in more powerful and continuous inhibition of platelet aggregation (Bavry, et al., 2007).

1.5.2 Thromboxane

Thromboxane A2, produced via the arachidonic acid (AA) pathway, is an important platelet activation pathway (Figure 1-4). This pathway activates the TP-receptor and induces further platelet activation. Activation of platelets and

platelet bridging encourage production of thrombin and procoagulant activity and the formation of stable platelet plugs (Andrews and Berndt, 2004). Aspirin, a drug widely used in treating cardiovascular diseases, inhibits platelet aggregation via inactivation of cyclooxygenase (COX) and thus resulting in the blockage of production of TxA2.

As shown in Figure 1-4, 11-dehydro-thromboxane B2 (11-dhTXB2) is one of the product produced from the AA pathways and it is a stable metabolite of TxA2. 11-dhTxB2 is useful in testing the extent of TxA2 inhibition for aspirin resistance and also in monitoring platelet activation for those who did not taking aspirin (Catella, et al., 1986; Eikelboom, et al., 2002; Baigent, et al., 2009). 11-dhTxB2 levels can be measured in plasma or urine. A study (McConnell, et al., 2001) found that 11-dhTxB2 levels are elevated during acute ischaemic stroke, especially in those who did not taking aspirin. A high concentration of 11-dhTxB2 level in patients treated with aspirin therapy indicate an inadequate inhibition of TxA2 synthesis. Furthermore, higher concentrations of 11-dhTXB2 level have been associated with an increased risk of cerebral infarction, myocardial infarction, saphenous vein graft thrombosis after coronary artery bypass graft surgery, and cardiovascular death (Eikelboom, et al., 2002; Eikelboom, et al., 2008; Gluckman, et al., 2011).

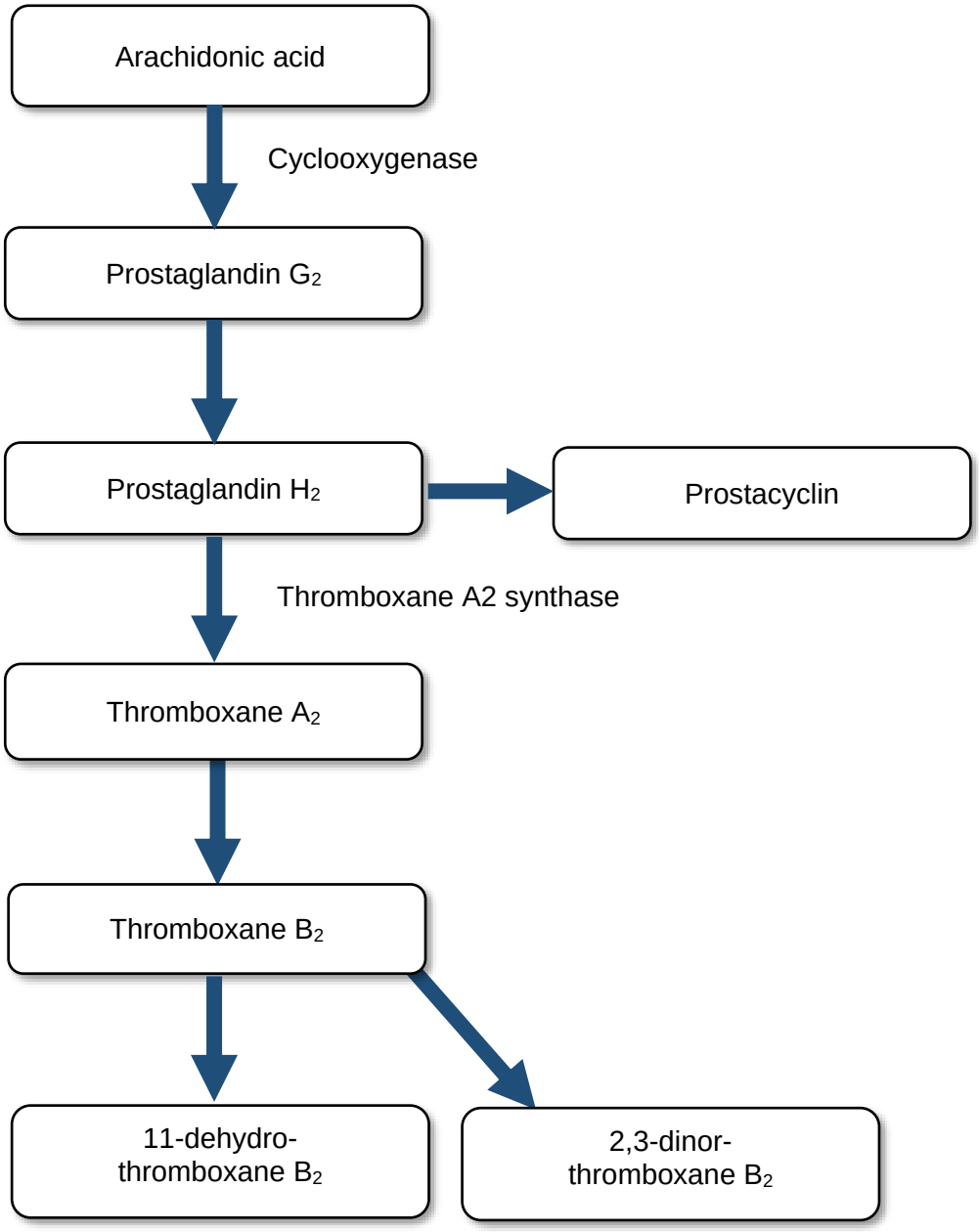


Figure 1-4: Arachidonic acid thromboxane pathway.

[Ref: Born and Patrono (2006)]

1.5.3 Antiplatelet withdrawal and the rebound phenomenon

There is evidence of increased risk of cardiovascular events following antiplatelet cessation. There are suggestions that this event maybe due to a rebound in platelet activity (Lordkipanidze, et al., 2009; Sambu, et al., 2011a; Sambu, et al., 2011b). Lordkipanidze, et al. (2009) has proposed that suppression of TxA2 by aspirin might lead to higher production of TxA2 than normal and it can cause rebound platelet aggregation once aspirin is discontinued. Similarly, while on clopidogrel, the $P2Y_{12}$ receptor increase in expression and sensitivity, thus, once discontinued, resulting in more efficiently activation of GP IIb/IIIa and cause an increase in platelet aggregation.

There is clinical evidence that cardiovascular events increased when changing from dual to mono antiplatelet therapy. While on DAPT, such as the combination of aspirin and clopidogrel, both pathways would be affected i.e. AA pathway and ADP pathway, as discussed earlier. There is evidence that clopidogrel influences AA pathways, thus resulting more effective inhibition platelet aggregation by aspirin (Frelinger, et al., 2006; Shankar, et al., 2006; Armstrong, et al., 2009; Armstrong, et al., 2010). Therefore, it is suggested that after discontinuation of clopidogrel, the synergistic effect will be lost and resulting in rebound attenuation of the antiplatelet effect of aspirin (Sambu, et al., 2011b).

With all these theories of rebound effect, there are studies done to support these theories. Diehl, et al. (2011) found levels of ADP increased at 2 weeks after clopidogrel cessation. Mylotte, et al. (2011) found an increased in platelet aggregation response to ADP and epinephrine when compared before and one week after cessation of clopidogrel. On the other hand, studies by Sambu, et al. (2011a) and Djukanovic, et al. (2011) found no evidence to support these theories. However, most of these studies do not include any measurement before the antiplatelet therapy.

1.6 Antiplatelet therapy

Platelets play an important role in haemostasis and thrombosis. There are many studies to support that inhibition of platelet aggregation significantly reduce mortality and morbidity in ischaemic atherothrombotic events (Gent, et al., 1996; Chen, et al., 1997; Sandercock, et al., 2014). Therefore, antiplatelet is a key in pharmacological treatment and prevention of CHD and cerebrovascular disease. The current antiplatelet agents available include acetylsalicylic acid (aspirin); thienopyridines (ticlopidine, clopidogrel, and prasugrel); reversible $P2Y_{12}$ receptor antagonists (ticagrelor, cangrelor) and dipyridamole. In this section, we are going to discuss the background of antiplatelet development, their mechanism of action, their indications and their common side effects.

1.6.1 Aspirin

The first antiplatelet drug is acetylsalicylic acid or commonly known as aspirin. Aspirin was first developed in 1897 as an analgesic and antipyretic agent (Miner and Hoffhines, 2007). In 1968, Weiss, et al. (1968) found that aspirin may prolong bleeding time and inhibited platelet aggregation and subsequently few years later others (Smith and Willis, 1971; Hamberg, et al., 1975; Roth and Majerus, 1975) had found the molecular mechanism of action of aspirin. Since the first introduction of antiplatelet, several new antiplatelet agents have been developed and marketed (Figure 1-5).

Antiplatelet drugs prevent thrombus formation and reduce platelet aggregation. Mechanisms of the platelet activation site and site of action of antiplatelet agents are shown in Figure 1-6. Aspirin has shown irreversible antiplatelet activity by reducing production of TxA2 through inhibition of the enzyme cyclooxygenase 1 (COX-1) (Roth and Majerus, 1975; Catella-Lawson, et al., 2001) and thus, inhibits thrombus formation and decreases platelet aggregation. Aspirin as antiplatelet therapy is used to prevent and reduce the risk of cardiovascular events. Aspirin is the most prescribed drug in antiplatelet therapy and cheaper than any other antiplatelet.

There are many trials have evaluated the effects of aspirin alone and in combination with $P2Y_{12}$ inhibitor in patients with ACS. In patients with acute myocardial infarction and patients with previous myocardial infarction, antiplatelet therapy, aspirin reduced the outcome of any vascular event compared to placebo (Baigent, et al., 2002; Baigent, et al., 2009). However, coronary lesions behave like unstable plaques and dual antiplatelet combination (e.g. aspirin plus clopidogrel) has proven to reduce cardiovascular death, nonfatal MI, or stroke compared to aspirin alone in non-ST elevation ACS (Yusuf, et al., 2001).

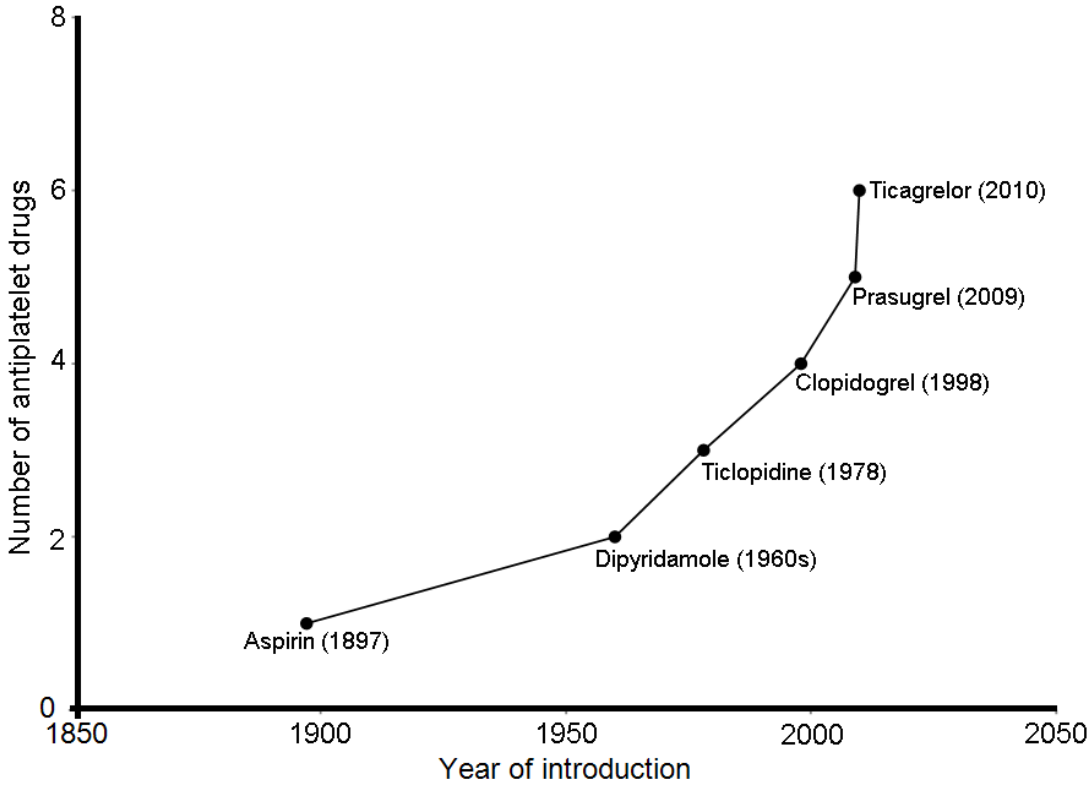


Figure 1-5: Year of introduction of antiplatelet drugs to the clinical management of cardiovascular disease.

[Ref: Sugidachi, et al. (2000); Sugidachi, et al. (2001); van Giezen and Humphries (2005); Born and Patrono (2006); Miner and Hoffhines (2007); Maffrand (2012)]

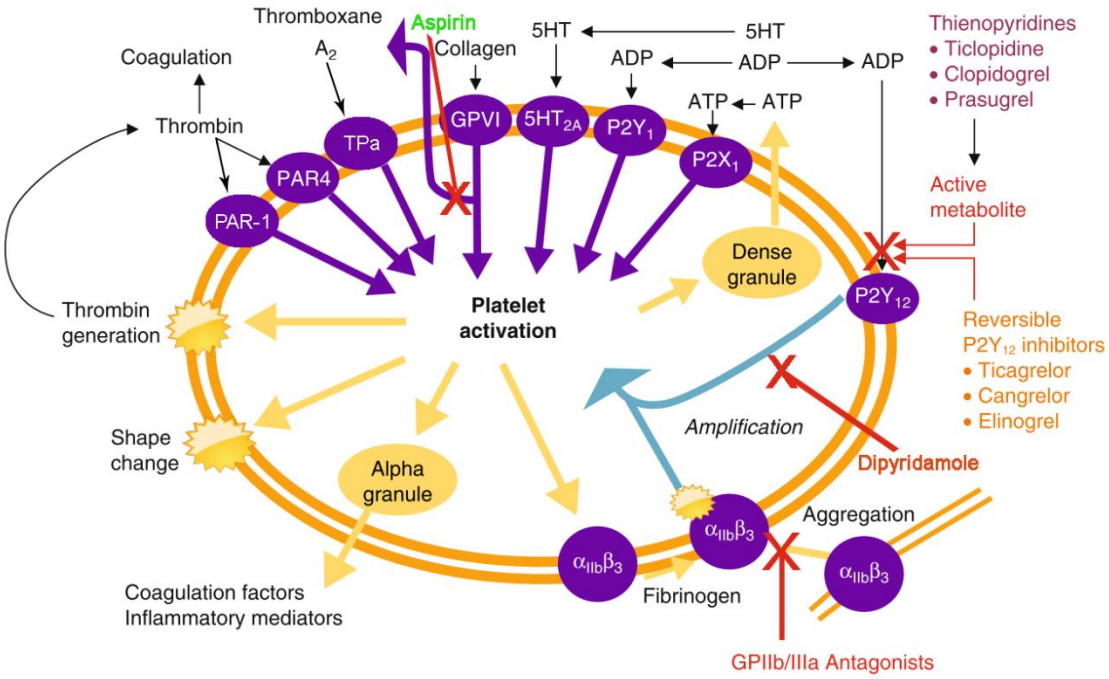


Figure 1-6: Platelet activation targets for antiplatelet agents.

ADP=adenosine diphosphate, ATP=adenosine triphosphate, GP=glycoprotein, PAR=protease-activated receptor, TPa=thromboxane receptor protein antagonist, 5HT=serotonin, 5HT2A=serotonin 2A receptor, $\alpha_{IIb}\beta_3$=GPIIb/IIIa. Adapted from Storey (2011) and Angiolillo (2012). (Permission obtained from Springer, License Number 3957510629094)

Aspirin has been proven to reduce recurrence stroke among patients with a recent stroke or TIA (Farrell, et al., 1991; Altman, et al., 1994). In a meta-analysis conducted by Johnson, et al. (1999), aspirin reduces the risk of stroke by 15% compared with placebo in patients with previous TIA or stroke (risk ratio (RR), 0.85; 95% CI, 0.77-0.94). Another study by The SALT Collaborative Group (1991) also found aspirin alone reduced recurrent stroke or death in patients with TIA or minor ischaemic stroke compared with placebo. A time-course analysis of randomized trials by Rothwell, et al. (2016) found that aspirin reduces the six weeks risk of recurrent ischaemic stroke by 60% (HR, 0.42; 95% CI, 0.32-0.55) compared with controls and reduces disabling or fatal ischaemic stroke by 70% (HR, 0.29; 95% CI, 0.20-0.42). In the first six weeks after randomization in trials in secondary prevention after TIA and ischaemic stroke, patients treated with aspirin shifted to better functional outcome (OR, 0.42; 95% CI, 0.26-0.70) (Rothwell, et al., 2016).

Antiplatelet therapy is indicated in patients undergone coronary artery bypass grafting (CABG) to prevent major complication such as graft occlusion and to prevent long-term cardiovascular events. Aspirin is essential to prevent platelet activation and should be initiated before undergoing surgery and continue long-term therapy post-CABG (Baigent, et al., 2002; Kulik, et al., 2009; O'Gara, et al., 2012). In a meta-analysis of 17 randomized trials, aspirin was significantly reduced the odds of graft occlusion compared with placebo (odds ratio (OR), 0.60; 95% CI, 0.51–0.71; p<0.0001) (Fremes, et al., 1993).

1.6.2 Dipyridamole

Dipyridamole was introduced in the early 1960s as a coronary vasodilator (Born and Patrono, 2006). Bunag, et al. (1964) found that dipyridamole inhibited adenosine uptake by platelet on platelet aggregation and soon few more studies (Emmons, et al., 1965; Elkeles, et al., 1968) found that dipyridamole inhibited platelet aggregation. Dipyridamole was then developed as an antithrombotic agent.

Dipyridamole acts by increase local adenosine concentration and also inhibits intracellular phosphodiesterase (PDE) enzyme. By these two actions, intracellular cyclic adenosine monophosphate (cAMP) concentration increased and prevent platelet activation and aggregation (Harker and Kadatz, 1983). Dipyridamole is a prodrug and irreversible inhibit PDE.

1.6.3 Ticlopidine

Ticlopidine was first marketed in France in 1978 but the drug was found few years earlier by Dr Ferdinand Eloy. In 1972, he was looking for new anti-inflammatory drugs related to Tinoridine, a thienopyridine with anti-inflammatory and analgesics property. While he was testing these new compounds, none of the new compounds had anti-inflammatory or analgesic effect. However, some of these thienopyridine compounds showed antiplatelet and antithrombotic properties. This is where they found ticlopidine, one of the most active thienopyridine compound (Maffrand, 2012).

Ticlopidine is selective and irreversibly inhibits the $P2Y_{12}$ receptor and prevent platelet activation. Ticlopidine is a prodrug and once absorbed in the intestine, they metabolized in the liver to form active metabolites (Savi and Herbert, 2005). However, the usage of ticlopidine was limited because of severe haematological disorders and life threatening side effects, including leucopenia, neutropenia, thrombocytopenia, agranulocytosis, pancytopenia and thrombotic thrombocytopenic purpura. This led to the development of clopidogrel with an improved safety profile.

Ticlopidine has been evaluated in three large trials of patients with cerebrovascular disease. In CATS (Canadian American Ticlopidine Study) trial, the risk of stroke, MI or vascular death was lowered in ticlopidine group compare to placebo (10.8% vs 15.3% per year; relative risk reduction (RRR), 30.2%; 95% CI, 7.5-48.3%) in patients who had sustained a thromboembolic stroke (Gent, et al., 1989). In another trial, ticlopidine has been found to be superior to aspirin (Hass, et al., 1989). Due to ticlopidine life-threatening side effect, ticlopidine is rarely used in current clinical practice.

1.6.4 Clopidogrel

Due to the life-threatening side effect of ticlopidine, the researchers tried to look for more stable and powerful antiplatelet effect. They synthesized more than thousand ticlopidine analogues, and found dextrogyre (S)-isomer (clopidogrel) which is more active and better tolerated than ticlopidine (Maffrand, 2012). Clopidogrel was launched in 1998 after 10 years development.

Clopidogrel has a similar mechanism of action as ticlopidine. It is selectively and irreversibly inhibit the $P2Y_{12}$ receptor and prevent platelet activation. Clopidogrel is also a prodrug where it needs to metabolize in the liver to form active metabolites (Savi and Herbert, 2005). Clopidogrel used was limited to high inter-individual variability and potential of drug-drug interaction. However, clopidogrel is preferred over ticlopidine, as clopidogrel has better tolerability and enhanced safety (Bertrand, et al., 2000).

There are many data to support the clinical benefit of clopidogrel in the treatment of CVD. For example, in clopidogrel versus aspirin in patients at risk of ischemic events (CAPRIE) study, a randomized trial, to evaluate the efficacy of clopidogrel versus aspirin monotherapy in the secondary prevention of atherosclerotic vascular diseases. This study involved 19,185 patients observed that patients treated with clopidogrel had an 8.7% reduction in the relative risk of ischaemic stroke, MI or vascular death compared with aspirin (p=0.043) (Gent, et al., 1996).

In the clopidogrel for high atherothrombotic risk and ischemic stabilization, management, and avoidance (CHARISMA) trial, a prospective, multicentre, randomized, double-blind, placebo-controlled study, compared the efficacy and safety of clopidogrel compared with placebo in aspirin treated patients. The combination of clopidogrel and aspirin demonstrated similar efficacy to aspirin among patients at high risk for a cardiovascular event in reducing MI, stroke or death (RR, 0.93; 95% CI, 0.83-1.05; p=0.22) (Bhatt, et al., 2006).

Clopidogrel, when given together with aspirin and fibrinolytic therapy has been shown to reduce the rate of death or re-infarction without major increased bleeding risk following ST-elevation MI (Chen, et al., 2005; Sabatine, et al., 2005b). Further, in the CURE trial, patients who presented within 24 hours after the onset of ACS were randomised to receive clopidogrel or placebo in addition to aspirin for 3 to 12 months. Compared to placebo, patients who were treated with clopidogrel had lower rates of death from cardiovascular causes, non-fatal MI or stroke (9.3 % vs 11.4 % in placebo) (Yusuf, et al., 2001).

Intracoronary thrombosis is likely to occur following PCI, thus, aspirin and $P2Y_{12}$ inhibitors has been used to reduce the risk of stent thrombosis (Schomig, et al., 1996; Bertrand, et al., 1998; Leon , et al., 1998). The combination of aspirin and ticlopidine has been shown to reduce the bleeding rates and subacute stent occlusion compared with an oral anticoagulant (Bertrand, et al., 1998). Few years later, another study by Bertrand, et al. (2000) demonstrated that clopidogrel and ticlopidine in combination with aspirin have similar efficacy in reducing cardiac events and the safety of clopidogrel is superior compared with ticlopidine. Recently, there is evidence that clopidogrel pre-treatment before

PCI significantly decreased the incidence of cardiac events (Mehta, et al., 2001; Sabatine, et al., 2005a). Major study findings of antiplatelet therapy for ACS are summarized in Table 1-4.

For ACS patients treated with CABG, the combination of aspirin plus clopidogrel had a lower rate of graft occlusion (RR, 0.59; 95% CI, 0.43–0.82; p=0.02) and in-hospital or 30-day mortality (0.8% vs 1.9%; p<0.0001) compared to aspirin alone (Deo, et al., 2013). In the TRITON-TIMI 38 trial, 346 CABG patients who were treated with prasugrel had a lower rate of death compared to patients treated with clopidogrel (adjusted OR, 0.26; 95% CI, 0.08–0.85; p=0.025) (Smith, et al., 2012).

Clopidogrel monotherapy is indicated for secondary prevention of stroke. In the prevention regimen for effectively avoiding second strokes (PRoFESS) trial, the efficacy and safety between clopidogrel versus aspirin plus extended-release dipyridamole regimen were compared. No difference was found in the risk of stroke recurrence between clopidogrel and aspirin plus extended-release dipyridamole (ERDP) (HR, 1.01; 95% CI, 0.92-1.11) (Sacco, et al., 2008).

In the clopidogrel in high-risk patients with acute non-disabling cerebrovascular events (CHANCE) study, the combination of aspirin and clopidogrel versus aspirin alone were compared among patients with TIA or minor ischaemic stroke. In the first 90 days, aspirin plus clopidogrel group had lower risk of stroke compared those in the aspirin group (8.2% vs 11.7%; hazard ratio (HR), 0.68; 95% CI, 0.57-0.81; p<0001) without increased risk of bleeding (Wang, et al., 2013). Major study findings of antiplatelet therapy following ischaemic stroke are summarized in Table 1-5. On the other hand, clopidogrel has become another reasonable option for those who is allergic to aspirin or unable to tolerate combination aspirin and dipyridamole.

1.6.5 Prasugrel

Prasugrel was found in 1993 by Japanese scientists. It is the third generation of thienopyridine. The group tested a series of hydropyridine compound and found some of the compound showed antithrombotic activities (Koike, et al., 1993).

They found prasugrel which is more potent than clopidogrel (Sugidachi, et al., 2000; Sugidachi, et al., 2001). Prasugrel was launched in 2009 and approved by the United State Food and Drug Administration (US FDA). The mechanism of action of prasugrel is similar to other thienopyridine.

Prasugrel has similar efficacy and risks of bleeding over clopidogrel in patients with unstable angina or NSTEMI treated medically. In TRILOGY-ACS trial, compared with clopidogrel, prasugrel therapy was not associated with significantly lowered rates of cardiovascular death, MI or stroke in 7243 with unstable angina or MI who did not undergo PCI (Roe, et al., 2012).

Prasugrel is another proven antiplatelet used for the treatment of ACS after PCI. In the TRITON-TIMI 38 trial, prasugrel was compared with clopidogrel among aspirin treated patients with ACS scheduled for PCI. The rates of ischaemic complications were reduced in prasugrel treated group but the risk of major bleeding was increased (Wiviott, et al., 2007).

1.6.6 Ticagrelor

Ticagrelor, the first reversibly-binding oral ADP receptor antagonist, was launched in 2010. It was started when investigators looking for an analogue of ATP, a compound known to inhibit the ADP-induced platelet aggregation. A series of potent $P2Y_{12}$ receptors' antagonist was identified and discovered cangrelor. However, cangrelor is intravenous, short-acting and no clinical efficacy was found. Further development of $P2Y_{12}$ receptors antagonist lead to identification of selective $P2Y_{12}$ receptor antagonist, ticagrelor (van Giezen and Humphries, 2005).

Ticagrelor is a non-thienopyridine agent. It is reversibly inhibit the $P2Y_{12}$ receptor and prevent platelet aggregation (Husted and van Giezen, 2009). Ticagrelor is not a prodrug, thus, it has a rapid onset of action that can be achieved within 30 minutes following the loading dose (Gurbel, et al., 2009). Ticagrelor has the shortest duration of the antiplatelet effect following cessation (Gurbel, et al., 2009) and it has no genetic variants of the CYP2C19 gene compared with clopidogrel (Tantry, et al., 2010).

Ticagrelor, in combination with aspirin is also indicated for the treatment of ACS. The PLATO trial, the efficacy and safety of ticagrelor and clopidogrel was evaluated. The findings showed that among ACS patients treated with aspirin, ticagrelor significantly reduced the rate of ischaemic complications compared with clopidogrel (9.8% vs 11.7%; HR, 0.84; 95% CI, 0.77-0.92) (Wallentin, et al., 2009a).

Ticagrelor, co-administered with low-dose aspirin is also indicated for the treatment of post-ACS after PCI. In the PLATO trial, among 11,289 patients who received a stent and treated with aspirin, ticagrelor versus clopidogrel significantly reduced the rate of stent thrombosis (2.9% vs 3.8%; HR, 0.77; 95% CI, 0.62-0.95; p=0.01) (Wallentin, et al., 2009a). With this new evidence, ticagrelor is recommended over clopidogrel in post-ACS patients with PCI.

The use of ticagrelor in ACS patients with CABG was supported by the post-hoc analysis of 1261 patients who underwent CABG within 7 days of receiving study drug treatment in PLATO. In this subgroup analysis, the occurrence of cardiac events at 1 year was similar in ticagrelor and clopidogrel (HR, 0.84; 95% CI, 0.60-1.16; p=0.29). However, total mortality for ticagrelor was decreased compared with clopidogrel (4.7% vs 9.7%; HR, 0.49; 95% CI 0.32-0.77; p<0.01) (Held, et al., 2011).

Table 1-4: Key trials in antiplatelet therapy after ACS.

Trial	Details	Comparison	Number of events/patients		Effect size
			Intervention group	Reference group	
NSTEMI / Unstable angina					
ARMYDA-2 (Patti, et al., 2005)	USA/NSTEMI (n = 255)	Antiplatelet (600mg vs 300mg loading dose of clopidogrel) and risk of death, MI or target vessel revascularization	High loading dose 5/126	Conventional loading dose 16/129	p-value=0.041
CURE (Yusuf, et al., 2001)	USA/NSTEMI (n = 12,562)	Antiplatelet (aspirin + clopidogrel vs aspirin) and risk of MI, stroke or CV death	Aspirin + clopidogrel 582/6259	Aspirin + placebo 719/6303	RR=0.80, 95% CI: 0.72-0.90
TRILOGY-ACS (Roe, et al., 2012)	USA/NSTEMI (n=7243)	Antiplatelet (aspirin + prasugrel vs aspirin + clopidogrel) and risk of MI, stroke or CV death	Aspirin + prasugrel 364/3620	Aspirin + clopidogrel 397/3623	HR=0.91, 95% CI: 0.79-1.05
STEMI					
CLARITY (Sabatine, et al., 2005b)	STEMI (n=3,491)	Antiplatelet (aspirin + clopidogrel vs aspirin) and risk of TIMI flow grade 0 or 1, MI or death	Aspirin + clopidogrel 262/1752	Aspirin + placebo 377/1739	OR=0.64, 95% CI: 0.53-0.76
COMMIT (Chen, et al., 2005)	STEMI (n=45,852)	Antiplatelet (aspirin + clopidogrel vs aspirin) and risk of death, MI or stroke	Aspirin + clopidogrel 2121/22961	Aspirin + placebo 2310/22891	OR=0.91, 95% CI: 0.86-0.97

ACS					
CURRENT-OASIS 7 (Mehta, et al., 2010)	ACS (n= 25,086)	Antiplatelet (aspirin + clopidogrel 150mg vs aspirin + clopidogrel 75mg) and risk of MI, stroke or CV death	Aspirin + clopidogrel 150mg 330/8560	Aspirin + clopidogrel 75mg 392/8703	Adjusted HR=0·86, 95% CI: 0.74-0.99
PLATO (Wallentin, et al., 2009a)	ACS (n= 18,624)	Antiplatelet (aspirin + ticagrelor vs aspirin + clopidogrel) and risk of MI, stroke or CV death	Ticagrelor 864/9333	Clopidogrel 1014/9291	HR=0.84, 95% CI: 0.77-0.92
Percutaneous coronary intervention					
ACCOAST (Montalescot, et al., 2013)	NTSEMI before PCI (n=4033)	Antiplatelet (aspirin + prasugrel vs aspirin) and risk of MI, stroke, urgent PCI or CV death	Aspirin + prasugrel 203/2037	Aspirin + placebo 195/1996	HR=1.02, 95% CI: 0.84-1.25
CLASSICS (Bertrand, et al., 2000)	After coronary stenting (n=1,020)	Antiplatelet (aspirin + clopidogrel vs aspirin + ticlopidine) and risk of major peripheral or bleeding complications, neutropenia, thrombocytopenia or early discontinuation of study drugs	Aspirin + clopidogrel 31/680	Aspirin + ticlopidine 31/340	RR=0.50, 95% CI: 0.31-0.81
CREDO (Steinhubl, et al., 2002)	Before coronary stenting (n=2,116)	Antiplatelet (aspirin + clopidogrel for 12 months vs aspirin + clopidogrel for 30 days) and risk of death, MI or stroke	Aspirin + clopidogrel for 12 months 89/1053	Aspirin + clopidogrel for 30 days 122/1063	RRR=26.9%, 95% CI: 3.9-44.4
FANTASTIC (Bertrand, et al., 1998)	After coronary stenting (n=439)	Antiplatelet vs anticoagulant and risk of subacute stent occlusion	Aspirin + ticlopidine 1/249	Anticoagulant + aspirin 8/236	OR=0.12, 95% CI: 0.01-0.91

ISAR (Schomig, et al., 1996)	After coronary stenting (n=257)	Antiplatelet vs anticoagulant and risk of MI, CV death, aorta coronary bypass surgery or repeated angioplasty	Ticlopidine 4/257	Phenprocoumon 16/260	RR=0.25, 95% CI: 0.06-0.77
PCI-CLARITY (Sabatine, et al., 2005a)	STEMI-PCI (n=1863)	Antiplatelet (aspirin + clopidogrel vs aspirin) and MI, CV death or stroke from PCI	Aspirin + clopidogrel 34/933	Aspirin + placebo 58/930	OR=0.54, 95% CI: 0.35-0.85
PCI-CURE (Mehta, et al., 2001)	NSTEMI-PCI (n=2658)	Antiplatelet (aspirin + clopidogrel vs aspirin) and risk of MI, CV death or urgent angioplasty	Aspirin + clopidogrel 59/1313	Aspirin + placebo 86/1345	RR=0.70, 95% CI: 0.50-0.97
PRAGUE-18 (Motovska, et al., 2016)	AMI with PCI (n=1230)	Antiplatelet (aspirin + ticagrelor vs aspirin + prasugrel) and risk of death, re-infarction, urgent revascularization, stroke, bleeding	Aspirin + ticagrelor 24/596	Aspirin + prasugrel 25/634	OR=0.98, 95% CI: 0.55-1.73
TRITON-TIMI 38 (Wiviott, et al., 2007)	Moderate to high risk ACS scheduled for PCI (n=13,608)	Antiplatelet (aspirin + prasugrel vs aspirin + clopidogrel) and risk of MI, stroke or CV death	Aspirin + prasugrel 643/6813	Aspirin + clopidogrel 781/6795	HR=0.81, 95% CI: 0.73-0.90

ACCOAST=Pre-treatment at the Time of Diagnosis in Patients with Non-ST Elevation Myocardial Infarction, ACS=acute coronary syndrome, ARMYDA=Atorvastatin for Reduction of MYocardial Damage during Angioplasty, CI=confidence interval, CLARITY= Clopidogrel as Adjunctive Reperfusion Therapy, CLASSICS=CLopidogrel ASpirin Stent International Cooperative Study, COMMIT=ClOpidogrel and Metoprolol in Myocardial Infarction Trial, CREDO=Clopidogrel for the Reduction of Events During Observation, CURE=Clopidogrel in Unstable Angina to Prevent Recurrent Events, CURRENT-OASIS 7= Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events–Seventh Organization to Assess Strategies in Ischemic Syndromes, CV=cardiovascular, HR=hazard ratio, ISAR= Intracoronary Stenting and Antithrombotic Regimen, MI=myocardial infarction, NSTEMI=non-ST-elevation MI, OR=odds ratio, PCI=Percutaneous coronary intervention, PLATO= Platelet Inhibition and Patient Outcomes, RR=relative risk, STEMI=ST-elevation MI, TRILOGY-ACS= Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes, TRITON-TIMI= Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction, USA=unstable angina.

Table 1-5: Key trials in antiplatelet therapy after ischaemic stroke.

Trial	Details	Comparison	Number of events/patients		Effect size
			Intervention group	Reference group	
CATS (Gent, et al., 1989)	Thromboembolic stroke (n=1072)	Antiplatelet (ticlopidine vs placebo) and risk of stroke, MI, or vascular death	Ticlopidine 74/525	Placebo 118/528	RRR=30.2%, 95% CI: 7.5-48.3
CHANCE (Wang, et al., 2013)	TIA or minor stroke (n=5,170)	Antiplatelet (aspirin + clopidogrel vs aspirin) and risk of stroke	Aspirin + clopidogrel 212/2584	Aspirin alone 303/2586	HR=0.68. 95% CI: 0.57-0.81
EARLY (Dengler, et al., 2010)	Acute ischaemic stroke, NIHSS≤20 (n=543)	Antiplatelet (early aspirin + dipyridamole vs aspirin + dipyridamole after 7 days aspirin) and risk of vascular adverse events and death	Early aspirin + dipyridamole 28/283	Aspirin + later dipyridamole 38/260	HR=0·73, 95% CI: 0.44-1.19
ESPRIT (Halkes, et al., 2006)	TIA or minor stroke (n=2,739)	Antiplatelet (aspirin + dipyridamole vs aspirin) and risk of death from all vascular causes, stroke, MI or major bleeding	Aspirin + dipyridamole 173/1363	Aspirin alone 216/1376	HR=0.80, 95% CI: 0.66-0.98
ESPS-2 (Diener, et al., 1996)	Recent IS or TIA (n=6602)	Antiplatelet (aspirin + dipyridamole vs placebo) and risk of death and/or stroke	Aspirin + dipyridamole 286/1650	Placebo 378/1649	OR=0.71, 95% CI: 0.59-0.84

FASTER (Kennedy, et al., 2007)	TIA or minor stroke (n=392)	Antiplatelet (aspirin + clopidogrel vs aspirin) and risk of stroke	Aspirin + clopidogrel 14/198	Aspirin alone 21/194	RR=0.70, 95% CI: 0.3-1.2
IST (International Stroke Trial Collaborative Group, 1997)	IS (n=19,435)	Antiplatelet (aspirin vs placebo) and risk of any cause (stroke, CHD, PE, ECH, vascular or non-vascular cause)	Aspirin 872/9720	Placebo 909/9715	Event prevented 4 per 1000 (SD 4)
MATCH (Diener, et al., 2004)	Recent IS or TIA (n=7,599)	Antiplatelet (aspirin + clopidogrel vs clopidogrel) and risk of MI, stroke, vascular death or rehospitalisation for acute ischaemia	Aspirin + clopidogrel 596/3797	Clopidogrel alone 636/3802	RRR=6.4%, 95% CI: -4.6-16.3
PROFESS (Sacco, et al., 2008)	Recent IS (n=20,332)	Antiplatelet (aspirin + dipyridamole vs clopidogrel) and risk of first recurrence of stroke	Aspirin + dipyridamole 916/10,181	Clopidogrel 898/10,151	HR=1.01, 95% CI: 0.92-1.11
SOCRATES (Johnston, et al., 2016)	High risk TIA or non-severe stroke (n=13199)	Antiplatelet (ticagrelor vs aspirin) and risk of stroke, MI, death	Ticagrelor 442/6589	Aspirin 497/6610	HR=0.89, 95% CI: 0.78-1.01
SPS3 (The SPS3 Investigators, 2012)	Symptoma-tic lacunar infarct (n=3020)	Antiplatelet (aspirin + clopidogrel vs aspirin) and risk of recurrent stroke	Aspirin + clopidogrel 125/1517	Aspirin 138/1503	HR=0.92, 95% CI, 0.72-1.16

TASS (Hass, et al., 1989)	Cerebral/ retinal ischaemia or TIA (n=3069)	Antiplatelet (ticlopidine vs aspirin) and risk of recurrence of stroke	Ticlopidine	Aspirin	RRR=21%, 95% CI: 4-38

CATS=Canadian American Ticlopidine Study, CHANCE= Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events, CI=confidence interval, CHD=coronary heart disease, CV=cardiovascular, EARLY=Early treatment with aspirin plus extended-release dipyridamole for transient ischaemic attack or ischaemic stroke within 24 h of symptom onset, ECH=extracranial haemorrhage, ESPRIT= European/Australasian Stroke Prevention in Reversible Ischaemia Trial, ESPS-2=Second European Stroke Prevention Study, FASTER= Fast Assessment of Stroke and TIA to prevent Early Recurrence, HR=hazard ratio, IS=ischaemic stroke, IST=International Stroke Trial, MATCH=Management of ATherothrombosis for Continued Health, MI=myocardial infarction, NIHSS=National Institutes of Health Stroke Scale, OR=odds ratio, PE=pulmonary embolism, PRoFESS=Prevention Regimen For Effectively avoiding Second Strokes, RRR=relative risk reduction, SD=standard deviation, SOCRATES=Acute Stroke or Transient Ischaemic Attack Treated with Aspirin or Ticagrelor and Patient Outcomes, SPS3=Secondary Prevention of Small Subcortical Strokes, TASS=Ticlopidine ASpirin Stroke trial, TIA=transient ischaemic attack.

1.7 Current antiplatelet therapy guidelines

Antiplatelet therapy is a cornerstone of treatment for patients with atherosclerotic vascular disease. We have discussed pathophysiology of ACS and ischaemic stroke in the previous section and we understood that these events are triggered by rupture of atherosclerotic plaque which results in platelet activation and formation of thrombus. Therefore, therapeutic platelet inhibition is a crucial aspect of ACS and stroke management and secondary prevention. Antiplatelet therapy is also indicated for secondary prevention in patients discharged from hospital following post–PCI, and post–CABG, TIA and patients with peripheral arterial disease (PAD).

1.7.1 ACS and PCI

In patients presented or suspected with STEMI, the National Institute for Health and Care Excellence (NICE) and Scottish Intercollegiate Guidelines Network (SIGN) guidelines recommend a loading dose of aspirin 300mg in all patients and followed by aspirin 75mg daily as maintenance therapy and continued indefinitely (National Institute for Health and Care Excellence, 2013; Scottish Intercollegiate Guidelines Network (SIGN), 2013a). In those patients to be treated with primary PCI, a loading dose of ticagrelor 180mg is recommended followed by maintenance therapy with 90mg twice daily for up to 12 months (National Institute for Health and Clinical Excellence, 2011).

In patients who had STEMI and treated with a bare metal stent (BMS) or drug eluting stent (DES), a loading dose of clopidogrel 300mg or 600mg is recommended. Clopidogrel 75mg daily is then continued for up to 12 months. On the other hand, in patients with STEMI and medically managed with or without thrombolytic agent, clopidogrel 75mg daily should be continued for at least one month and may consider to continue for up to 12 months (Chen, et al., 2005; Sabatine, et al., 2005b; National Institute for Health and Clinical Excellence, 2013). NICE guideline recommends prasugrel as a treatment of option in those to be treated for primary PCI, patients with stent thrombosis while on clopidogrel or patients who had diabetes (National Institute for Health and Clinical Excellence, 2014).

In patients with NSTEMI or unstable angina, initial treatment includes a loading dose aspirin 300mg and ticagrelor 180mg or clopidogrel 300mg (Yusuf, et al., 2001; Wallentin, et al., 2009a). A maintenance dose of aspirin 75mg daily should be continued indefinitely. It is recommended ticagrelor 90mg twice daily or clopidogrel 75mg daily continued as maintenance therapy for up to 12 months (National Institute for Health and Care Excellence, 2010; National Institute for Health and Clinical Excellence, 2010; National Institute for Health and Clinical Excellence, 2011).

For all patients with ACS, following hospital discharge, it is recommended to continue low dose aspirin for a long term without interruption. For patients allergic or intolerance of aspirin, clopidogrel is indicated as an alternative to aspirin (Gent, et al., 1996). The recommended duration of antiplatelet therapy for ACS patients with or without PCI are summarized in Table 1-6.

For ACS patients with obstruction coronary arteries, PCI is indicated to induce atherosclerotic plaque rupture and followed by the implantation of stent in the artery. PCI has been proven to reduce rates of recurrent angina, rehospitalisation, MI and death (Bavry, et al., 2006; Hoenig, et al., 2010). DAPT is prescribed at the time of procedure and following the placement of BMS or DES in order to prevent stent thrombosis (Mehta, et al., 2001; Steinhubl, et al., 2002).

Table 1-6: Recommended duration of antiplatelet therapy

Antiplatelet drug and dosage	Indication	Duration
Aspirin (300mg loading, then 75-150mg daily)	ACS (with or without PCI)	Long term
	TIA or ischaemic stroke	Long term (alone)
Clopidogrel (300-600mg loading, then 75mg daily)	Unstable angina/ NSTEMI (medically managed)	At least 1 month and up to 12 months
	Unstable angina/ NSTEMI (with PCI and BMS or DES)	At least 12 months
	Unstable angina/ NSTEMI (managed by CABG)	Minimum 1 months and up to 12 months
	STEMI (with or without fibrinolysis)	At least 1 month or up to 12 months
	STEMI (with PCI and BMS or DES)	At least 12 months
	TIA or ischaemic stroke	Long term (alone)
Prasugrel (60mg loading, then 10mg daily)	STEMI (with PCI and BMS or DES)	At least 12 months
Ticagrelor (180mg loading, then 90mg twice a day)	Unstable angina/ NSTEMI (moderate to high risk*)	Up to 12 months
	Unstable angina/ NSTEMI (medically managed)	Up to 12 months
	Unstable angina/ NSTEMI (with PCI and BMS or DES)	At least 12 months
	Unstable angina/ NSTEMI (managed by CABG)	At least 12 months
	STEMI (with PCI and BMS or DES)	At least 12 months
Dipyridamole ER (200mg twice a day)	TIA or ischaemic stroke	Long term (alone)

*as defined in ≥2 or more of (1) ischaemic ST changes on electrocardiogram; (2) positive biomarkers; or (3) 1 of the following: ≥60 years of age, previous MI or CABG, Coronary artery disease >50% stenosis in 2 vessels, previous ischaemic stroke, diabetes, peripheral arterial disease, or chronic renal dysfunction.
ACS=acute coronary syndrome, BMS=bare-metal stents, CABG= coronary artery bypass grafting, DES=drug-eluting stents, ER= extended-release, PCI=percutaneous coronary intervention, TIA=transient ischaemic attack. (Wallentin, et al., 2009a; Bell, et al., 2011; National Institute for Health and Clinical Excellence, 2011; O'Gara, et al., 2012; Scottish Intercollegiate Guidelines Network (SIGN), 2013a; Tanguay, et al., 2013; Amsterdam, et al., 2014)

1.7.2 Ischaemic stroke

Following TIA or non-cardioembolic stroke, patients should be treated with antiplatelet therapy. NICE and SIGN guidelines on the management of acute ischaemic stroke and TIA recommend that for patients who had a suspected TIA, either high or low risk, aspirin 300mg daily should be started immediately. Whereas for patients with acute ischaemic stroke, as soon as possible, aspirin 300mg should be given within 24 hours of symptom onset. Thereafter, aspirin should be continued for 2 weeks after the onset of stroke symptoms, at which time definitive long term antiplatelet therapy should be initiated (National Institute for Health and Clinical Excellence, 2008; Scottish Intercollegiate Guidelines Network (SIGN), 2008). An alternative antiplatelet agent should be given for those who is allergic or intolerant to aspirin.

In recent NICE and SIGN guidelines, clopidogrel is recommended as an option for patients diagnosed with ischaemic stroke. DAPT with aspirin and modified-release dipyridamole is recommended for those patients with TIA or patients in whom clopidogrel is contraindicated or not tolerated (National Institute for Health and Clinical Excellence, 2010; Scottish Intercollegiate Guidelines Network (SIGN), 2013b).

1.7.3 Peripheral artery disease (PAD)

Antiplatelet therapy is also recommended for vascular prevention in patients with PAD. There are two types of patients with PAD, asymptomatic and symptomatic patients. For patients with asymptomatic PAD, with either a bruit along the major blood vessel or reduced or absent peripheral pulsation with an ankle-brachial index (ABI) ≤ 0.9, low-dose aspirin may be considered. Aspirin is indicated for those with high risk of atherosclerotic risk factors and without risk for bleeding (Fowkes, et al., 2010). For patients with symptomatic PAD with evident IHD or cerebrovascular disease, antiplatelet therapy is as indicated for ACS or ischaemic stroke treatment, which have been discussed in the previous section. Whereas for patients without IHD or ischaemic stroke, low-dose aspirin or clopidogrel is recommended, providing the risk of bleeding is low (Berger, et al., 2009).

NICE guidelines on the management of lower limb PAD recommend aspirin 75mg daily (National Institute for Health and Clinical Excellence, 2012). The dose was as effective as higher doses. The NICE technology appraisal 210 (National Institute for Health and Clinical Excellence, 2010) recommends clopidogrel as an option for those with PAD. However, in treating patients with PAD, combination therapy of aspirin and clopidogrel should be used cautiously as the combination of both drugs had a higher risk of bleeding complications (Bhatt, et al., 2006).

1.7.4 Duration of antiplatelet therapy

According to NICE guidelines for management of ischaemic stroke and PAD, antiplatelet regimen should be continued for a long term in order to reduce the risk of recurrence event. Whereas, for ACS with or without PCI, after a period, DAPT is stopped and continue with mono antiplatelet therapy. The reason behind this is that the long term usage of DAPT beyond certain period does not lower the risk of major cardiovascular event and at the same time, it is associated with an increased risk of bleeding. Table 1-6 summarized the recommendation duration of DAPT in patients with ACS.

1.7.5 Adverse events of antiplatelet therapy

Antiplatelet therapy is associated with adverse events like gastrointestinal and non-gastrointestinal bleeding, intracranial haemorrhage (ICH) and allergic reactions (swelling of the lips, tongue or face, coughing, wheezing or shortness of breath and anaphylaxis). The risk of bleeding due to antiplatelet therapy is because antiplatelet inhibits platelet activation or aggregation pathways (Meadows and Bhatt, 2007). There are about 3% incidence of gastrointestinal tract bleeding in patients treated with antiplatelet (Alli, et al., 2011). Bleeding at puncture and surgery site is another common types of bleeding, especially in patients undergoing PCI or CABG surgery, with an incidence of 1-2% patients (Mehta, et al., 2001; Kastrati, et al., 2004; Kapetanakis, et al., 2006). However, among patients treated with antiplatelet therapy, ICH is rare but it is associated with increased death (Gent, et al., 1996; Yusuf, et al., 2001; Chen, et al., 2005; Sandercock, et al., 2014).

Aspirin is associated with an increased risk of bleeding that is mainly gastrointestinal and rarely intracranial when compared with placebo. Low-dose aspirin (<100mg) was associated with the lowest risk, and moderate doses (100-200mg) caused slightly high bleeding event rate, particularly in minor, gastrointestinal, stroke and total bleeding (Serebruany, et al., 2005a). Side effects of aspirin include allergic reaction and gastrointestinal complications. The risk of symptomatic ulcer was higher in aspirin user compared to non-users and increased with any doses as low as 75mg daily (García Rodríguez and Hernández-Díaz, 2004).

Incidence of ICH and gastrointestinal bleeding in clopidogrel is comparable with aspirin (Gent, et al., 1996). However, a combination of DAPT is associated with an increased risk of bleeding than those who received aspirin alone (Yusuf, et al., 2001). Other side effects of clopidogrel are gastrointestinal problems. Severe rash also associated with clopidogrel which was more frequent with clopidogrel than aspirin (Gent, et al., 1996). Neutropenia was rare with clopidogrel. Although prasugrel reduced the incidence of ischaemic events when compared with clopidogrel, prasugrel was found to associate with about 30% increase in the relative risk of bleeding (Wiviott, et al., 2007). Adverse reactions following prasugrel include hypersensitivity reaction including angioedema and thrombotic thrombocytopenic purpura .

On the other hand, ticagrelor has similar rates of total major bleeding or life-threatening or fatal bleeding when compared with clopidogrel (Wallentin, et al., 2009a; Cannon, et al., 2010). Ticagrelor is associated with dyspnea. Incidence of dyspnea in patients treated with ticagrelor was between 14% to 39% and it was reported higher in ticagrelor group compared with clopidogrel (Wallentin, et al., 2009b; Storey, et al., 2010).

Hematologic adverse reactions are a known adverse event caused by ticlopidine. Ticlopidine is associated with an incidence of neutropenia of up to 2.1% of ticlopidine-treated patients (Gent, et al., 1989). Other side effects of ticlopidine include diarrhea, nausea and vomiting and occur in 30% to 50% of patients (McTavish, et al., 1990).

Recently, there are doubts about the safety of discontinuation of DAPT in ACS population (Ho, et al., 2010; Charlot, et al., 2012), and this is discussed in details in the next section.

1.8 Can antiplatelet be better targeted?

Despite major improvements in antiplatelet therapy, ischaemic complications remain a concern after ACS and ischaemic stroke. Recently, there have been reports of increased risk of cardiovascular events from discontinuation of antiplatelet therapy (D'Ascenzo, et al., 2014). This cardiovascular event includes all-cause mortality, hospitalization for MI and/or stent thrombosis (Ho, et al., 2010; Mehran, et al., 2013). In this section, we are going to discuss the risk of cardiovascular event in patients, despite with antiplatelet therapy, in those who changed from DAPT to single antiplatelet and those who stopped or interrupted antiplatelet treatment.

1.8.1 Cardiovascular event despite antiplatelet therapy

Survivors of MI and ischaemic stroke are at increased risk of recurrent infarctions and recurrent ischaemic stroke. They have been prescribed with evidence based interventions for secondary prevention includes the use of antiplatelet therapy. However, there is evidence that the cardiovascular event still occurred despite the use of antiplatelet therapy in these populations.

In ACS patients, despite treatment with antiplatelet therapy, recurrent MI occurred. In a study by DÖnges, et al. (2001), 91% of the patients with recurrent MI had been prescribed with aspirin therapy previously. Later, a study by Thune, et al. (2011) also showed that in their recurrent MI sample, 89.2% of patients had been taking aspirin before the recurrent event.

Antiplatelet therapy indicated for acute ischaemic stroke or TIA are for a long term. Similarly, a cardiovascular event still occurred, whilst patients still taking antiplatelet therapy. In the recently published CHANCE study, at least 20% of patients had a history of previous stroke and 11% of patients had their index stroke whilst taking antiplatelet therapy (Wang, et al., 2013). In North Dublin

Stroke study, 34.8% patients in their sample were taking antiplatelet therapy before first-ever stroke (Kelly, et al., 2012). However, there was not mentioned any assessment on medication adherence before the hospital admission. At the same time, guidelines acknowledge that in patients who experience a stroke whilst taking antiplatelet therapy, there are no clinical trials to show whether switching antiplatelet agents decreases the risk of subsequent events (Furie, et al., 2011; Kernan, et al., 2014).

This evidence, has raised questions what are the possibilities that the events happened in these patients. There are many factors associated with antiplatelet response. There might be resistance or non-response to antiplatelet agent. Published data suggest that underdosing, poor compliance, poor drug absorption, drug–drug interaction and stress-induced generation enzyme cyclooxygenase (COX-2) in platelets or polymorphisms of COX-1 might be responsible for the insufficient response to aspirin (Michos, et al., 2006). The mechanism of aspirin and clopidogrel resistance are likely to be multifactorial and figure 1-7 summarised possible causes for aspirin and clopidogrel resistance.

Resistance to antiplatelet therapy might be one of the reasons behind this event. Resistance to clopidogrel and aspirin exists and studies have shown that aspirin or clopidogrel resistance is associated with an increased risk of cardiovascular events (Matetzky, et al., 2004; Mason, et al., 2005; Ozben, et al., 2011). Resistance to aspirin is common and may be present in 25 to 30% of patients (Alberts, et al., 2004; Hovens, et al., 2007). Studies by Bennett, et al. (2008), Boncoraglio, et al. (2009) and Ozben, et al. (2011) did not find any significant difference between aspirin-resistant and aspirin-sensitive patients with regard to demographics, clinical characteristics, and risk factors. At the same time, Karepov, et al. (2008) reported that patients with aspirin resistance had significantly increased level of triglycerides compared with aspirin-sensitive patients. Moreover, Jeon and Cha (2008) and Ozben, et al. (2011) found that patients with aspirin resistance had higher stroke severity scores and Englyst, et al. (2008) pointed out that patients with aspirin resistance is associated with more severe strokes.

The dose of aspirin is important to prevent aspirin resistance. Inadequate dosing due to poor gastrointestinal absorption maybe possible. There are studies found enteric-coated aspirin had insufficient and decreased antiplatelet effect compared to plain aspirin (Maree, et al., 2005; Cox, et al., 2006; Peace, et al., 2010). On the other hand, low-dose of aspirin has been found to reduce antiplatelet effect. Lee, et al. (2005) found aspirin dose less than 100mg/day is associated with a higher incidence of aspirin resistance in patients with coronary artery disease. Moreover, interaction of antiplatelet agent with other medications may reduce antiplatelet efficacy. For examples, between aspirin and nonsteroidal anti-inflammatory drugs (Catella-Lawson, et al., 2001); or clopidogrel and omeprazole (Sibbing, et al., 2009).

Another possible mechanism associates with antiplatelet resistance is increased platelet turnover. Previous studies suggested that patients with accelerated platelet turnover may have reduced antiplatelet effects (Kour, et al., 2006; Airee, et al., 2008; Zimmermann and Hohlfeld, 2008; Li, et al., 2009; Grove, et al., 2011). A study by Grove, et al. (2011) pointed out that smoking, diabetes and thrombopoietin levels were identified as significant predictors of platelet turnover. Synthesis of thromboxane A2 by COX-2 may be another possible cause of aspirin resistance but inhibition of COX-2 might need higher doses of aspirin (500mg/day) (Kour, et al., 2006; Airee, et al., 2008). Other than COX-2, platelets can be activated towards other aggregating factors such as erythrocyte induced platelet activation; cigarette smoking; or diabetic patients caused increased production of prostaglandin F2 (Kour, et al., 2006; Gupta and Casterella, 2007; Airee, et al., 2008; Zimmermann and Hohlfeld, 2008; Li, et al., 2009).

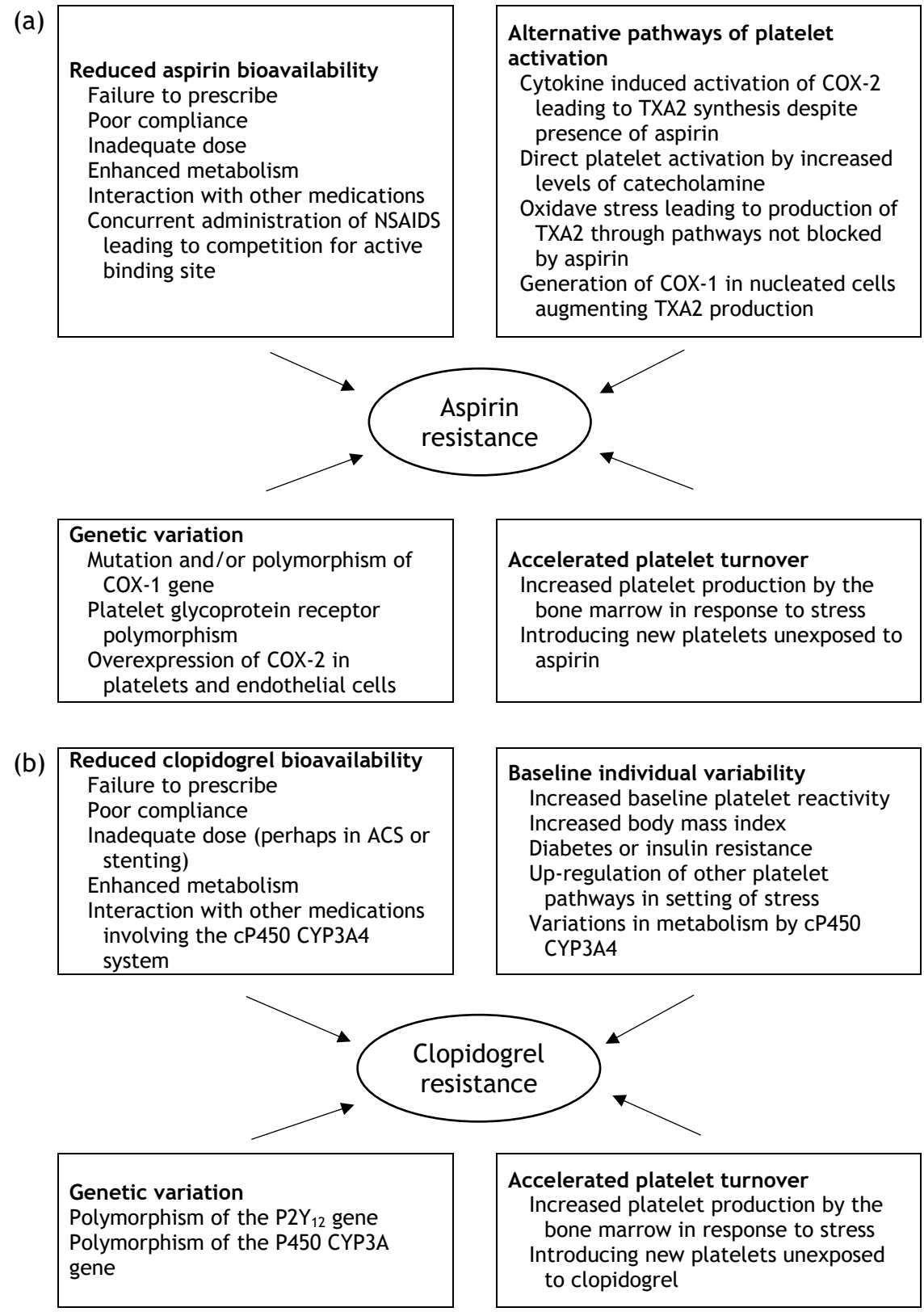


Figure 1-7: Proposed mechanisms for (a) aspirin and (b) clopidogrel resistance.

ACS=acute coronary syndrome; COX=cyclooxygenase; NSAIDs=nonsteroidal anti-inflammatory drugs; TXA2=thromboxane A2. (Permissions obtained from Elsevier, License Number 3742560329349)(Michos, et al., 2006)

Genetic polymorphisms involved in platelet activation may lead to less antiplatelet effect. For example, individuals with genetic variants of CYP2C19*2 and CYP2C19*3, had a decreased conversion of clopidogrel to its active metabolite (Hulot, et al., 2006; Mega, et al., 2009; Sofi, et al., 2011). Thus, they may experience a subsequent thrombotic event despite receiving clopidogrel (Kour, et al., 2006; Gupta and Casterella, 2007; Gurbel and Tantry, 2007; Airee, et al., 2008; Zimmermann and Hohlfeld, 2008; Bonello, et al., 2009; Li, et al., 2009). On the contrary, prasugrel may offer as an alternative in clopidogrel resistance as it is not activated in the liver (Gupta and Casterella, 2007; De Miguel, et al., 2008; Bonello, et al., 2009; Angiolillo, et al., 2010).

It is possible that patients who resistance to a certain antiplatelet agent should be switched to another class of antiplatelet. Furthermore, there are no trials to show switching antiplatelet agents after an acute event decreases the risk of subsequent events. Thus, we are going to answer this question by using acute stroke trial population.

1.8.2 Risks of changing from DAPT to single

Antiplatelet therapy is required after an ACS. DAPT typically using aspirin and an ADP receptor antagonist such as clopidogrel, is superior to use than a single agent in terms of reducing subsequent cardiovascular risk. There were patients who experience thrombotic events within the first 3 to 6 months after completed the recommended duration of DAPT.

There are reports supported the cardiovascular risk is increased in the period following cessation of DAPT and initiation of monotherapy. This has been reported in both medically and PCI-treated patients after ACS. In 2008, a study from the Veterans Health Administration (Ho, et al., 2008b) found the first 90 days after stopping clopidogrel were associated with an increased risk of cardiovascular events. Later on, another study by Ho, et al. (2010) found a higher risk of death or MI at day 0 to 90 days compared to day 91 to 180 days after stopping clopidogrel. They found the risk of death or MI in the 0-90-day interval after clopidogrel cessation was two times higher compared with later time intervals. The timing of the events suggests they are specific to clopidogrel discontinuation and there were no differences in terms of the type of stent

used. Further, they found no increase in event rate after stopping ACE inhibitors further suggesting a causal relationship with clopidogrel cessation rather than a general effect of discontinuing medications.

The evidence of cardiovascular event after DAPT discontinuation did not only observed in observational studies, but also observed in a systematic review and meta-analysis which has higher power and precision of estimates of treatment effects and exposure risks. In a systematic review and meta-analysis of 49,586 patients found that ACS patients undergoing PCI experienced more cardiovascular events (OR, 1.19; 95% CI, 1.07–1.32) than medically-treated patients (OR, 1.13; 95% CI, 0.95–1.35) if they stopped DAPT over 12 months (D'Ascenzo, et al., 2014). They concluded that this result was hypothesis generating and should be confirmed in randomized controlled trials.

There is one prospective observational study tried to address these clinical important issues. The Patterns of non-adherence to Antiplatelet Regimens In Stented patients (PARIS) study recently showed that the effect of DAPT cessation on cardiac risk after PCI may be related to the reason that DAPT was stopped. This study includes patients undergoing PCI with stent implantation and followed them up at 1, 6, 12 and 24 months after implantation. They found out that there was no excess risk if DAPT was stopped as planned post ACS. Where it was interrupted due to a planned procedure such as surgery there was similarly no increased risk. However, where DAPT was stopped due to bleeding or non-compliance, there was an increased risk (Mehran, et al., 2013).

In patients undergoing PCI, stent thrombosis is the common cardiovascular event occurred after discontinuation of DAPT. Iakovou, et al. (2005) found that premature DAPT discontinuation as an independent predictors of stent thrombosis. Similarly, Park, et al. (2006) found the incidence of stent thrombosis is significantly increased in patients with premature discontinuation of aspirin or clopidogrel or both. Later, Airoldi, et al. (2007) showed discontinuation of thienopyridine therapy was the predictors of stent thrombosis within the first six months.

It is clear that data showed cessation of DAPT therapy, may put patients at risk. This has raised demands what are the possibilities that events happened in these patients and a better understanding of this may allow strategies to mitigate this risk.

There are few factors associated with increased risk of cardiovascular events while changing from dual to single antiplatelet therapy. Duration of DAPT plays an important role to cause increased risk of cardiovascular event following DAPT discontinuation. Several studies (Airoldi, et al., 2007; Ho, et al., 2008b; Ho, et al., 2010; Mauri, et al., 2014) have established associations between duration of DAPT and risk of cardiovascular event. Duration of clopidogrel treatment less than 6 months was associated with increased risk of death or MI within 90 days after stopping clopidogrel (Ho, et al., 2010). Boggon, et al. (2011) found that the hazard of death or non-fatal MI was significantly higher among patients who discontinued clopidogrel compared with patients who continued to receive a clopidogrel prescription. However, some studies (Mauri, et al., 2014; Bulluck, et al., 2015) found that longer DAPT duration more than one year was associated with increased risk of bleeding.

Patients with ACS can be managed with medication regimens or with PCI. Medically-treated patients without other cardiac interventions were found to have higher risk of death or recurrent ACS within 90 days after discontinuation of clopidogrel (Ho, et al., 2008b; Ho, et al., 2010; Stephenson, et al., 2011). In addition, Stephenson, et al. (2011) found patients with BMS experienced a higher incidence of death or recurrent ACS compared with DES. In contrast, a recent systematic review and meta-analysis pointed out the risk of cardiovascular events was higher in patients receiving stents but not in medically-treated patients (D'Ascenzo, et al., 2014). However, the meta-analysis only included studies evaluating DAPT duration longer than 12 months.

Different modes of DAPT discontinuation also plays an important role in increased risk of thrombotic events following DAPT discontinuation. There were three modes of DAPT cessation studied by Mehran, et al. (2013) i.e. discontinuation, interruption and disruption. Between these three modes, disruptions of DAPT due to bleeding or non-compliance were associated with

increased risk of major adverse cardiovascular events (MACE) following DAPT withdrawal. On the other hand, this study found that discontinuation of DAPT by physician-guided was associated with lower MACE.

There are suggestions that the clustering of cardiovascular events occurs following DAPT cessation maybe due to rebound phenomena that associated with prothrombotic or proinflammatory response (Sambu, et al., 2011b). Previous studies have investigated the effect of clopidogrel discontinuation on inflammatory biomarkers (Wykrzykowska, et al., 2009), platelet reactivity (Sibbing, et al., 2010) and combination of inflammatory biomarker and platelet reactivity in diabetic population (Angiolillo, et al., 2006). Recently, Sambu, et al. (2011a) investigated the effect of clopidogrel withdrawal after one year DES PCI on arachidonic acid-induced platelet aggregation and ADP-induced platelet aggregation, and biomarkers of vascular inflammation, including soluble CD40 ligand (sCD40L), high-sensitivity C-reactive protein (hsCRP) and interleukin 6 (IL-6) at multiple time-points following clopidogrel cessation. There was a significant increased in ADP-induced platelet aggregation, arachidonic acid-induced platelet aggregation and a decreased in IL-6 at 1, 2 and 4 weeks; and a decreased hsCRP at 4 weeks following clopidogrel cessation. Table 1-7 summarises the key trials on mechanism occurs following DAPT cessation.

Table 1-7: Key trials on mechanism occurs following DAPT cessation.

Studies	Details	Comparison	Findings
Angiolillo, et al. (2006)	54 patients with diabetes, undergone PCI and treated with clopidogrel for 1 year	Within 1 month while patients on aspirin and clopidogrel vs 1 month after withdrawal (patients only on aspirin)	Significant increased in ADP-induced platelet aggregation and inflammatory biomarker (CRP)
Wykrzykowska, et al. (2009)	98 non-diabetic patients, following drug-eluting stents and treated with clopidgrel for 12 months	Last day on clopidogrel (baseline) vs 1,2,3 and 4 weeks after withdrawal	Significant increased in sCD40L biomarker at 4 time-points; significant increased in P-selectin at week 1 and 2; significant decreased in CRP at week 1, 2 and 3.
Sibbing, et al. (2010)	69 patients following DES were randomized to tapering group vs abrupt clopidogrel group	After study inclusion and at weeks 2,3 and 4 after randomization (while tapering clopidogrel dose or abrupt clopidogrel withdrawal); and weeks 5,6,7 and 8 (complete clopidogrel withdrawal for both groups)	Increased in ADP-induced platelet aggregation for both groups during weeks 5 to 8 (after complete clopidogrel withdrawal)
Sambu, et al. (2011a)	33 patients after one year DES PCI treated with clopidogrel for 1 year	At 4 weeks and 24 hours before clopidogrel withdrawal and at 24,48 hours, 1,2,4 weeks after clopidogrel withdrawal	Significant increased in ADP-induced platelet aggregation, AA-induced platelet aggregation and a decreased in IL-6 at 1, 2 and 4 weeks; and a decreased hsCRP at 4 weeks following clopidogrel cessation

AA=arachidonic acid; ADP=adenosine diphosphate; CRP=C-reactive protein; DES=drug-eluting stent; hsCRP=high-sensitivity C-reactive protein; IL=interleukin; PCI=percutaneous coronary intervention; sCD40L=soluble CD40 ligand.

1.8.3 Cardiovascular event after stopped or interrupted antiplatelet therapy

As listed in Figure 1-7, non-compliance or non-adherence to antiplatelet therapy may be one of the possible mechanisms of antiplatelet resistance and it is the most common cause of inadequate of antiplatelet effect (Schwartz, et al., 2008). About 50% of patients who were prescribed with aspirin or clopidogrel either discontinue taking their medication, or fail to adhere to their given dose at 1 year (Hamann, et al., 2003; Serebruany, et al., 2009; Herlitz, et al., 2010).

A clustering of cardiovascular events has been reported previously after aspirin cessation as well as after cessation of heparin. A study by Collet, et al. (2004) compared the rates of death and death or myocardial infarction between patients who were nonusers of oral antiplatelet, prior users of oral antiplatelet and recent withdrawers of oral antiplatelet. They found that, recent withdrawers (aspirin, n=70; ticlopidine, n=3) had a 2-fold increase in the rates of both death and death or myocardial infarction compared with prior users and nonusers. Kulkarni, et al. (2006) found the rates of patients who discontinued aspirin after one year was 18% of patients with established coronary artery disease. They found that the risk factors of withdrawal medication were older age, female, low levels of education, and unmarried status.

Withdrawal of antiplatelet therapy has been shown to have association with increased risk of stroke. The reported rates of aspirin discontinuation vary from less than 10% to almost 50% (Sud, et al., 2005; Lago, et al., 2006). One study found that among the 2197 cases of ischaemic stroke, 5.2% cases occurred within 60 days after antithrombotic withdrawal (Broderick, et al., 2011). In this study, stroke events were clustered mostly in the first 7 days after stopping medication and the antithrombotic medication was stopped for various reasons such as stopped by physicians for procedures, patient compliance, bleeding complications and cost.

In another study by García Rodríguez, et al. (2011), among 673 patients who had diagnosed with ischaemic stroke or TIA, 71.3% patients were taking aspirin on the day of event and 10% discontinued aspirin within 31-180 days before the

event. The group who discontinued aspirin, is associated with a 40% increased risk of stroke. This study shown that the events happened while patients still taking aspirin and within 6 months after aspirin withdrawal.

There are many reasons antiplatelet medication was stopped or interrupted. Antiplatelet therapy is commonly interrupted by physicians for procedures. In order to reduce the risk of bleeding during procedures, physicians make a decision to stop antiplatelet therapy, but this may expose patients to an increased risk for cardiovascular event and death (Iakovou, et al., 2005; Biondi-Zoccai, et al., 2006; Rossini, et al., 2011; Fleisher, et al., 2014). In contrast, if they decided to continue antiplatelet therapy in order to prevent cardiovascular event, this may cause increased risk of bleeding and need for blood transfusions during invasive procedures (Antiplatelet Trialists' Collaboration, 1994; Pulmonary Embolism Prevention (PEP) trial Collaborative Group, 2000; Burger, et al., 2005; Devereaux, et al., 2014). Figure 1-8 showed the balance between thrombotic and haemorrhagic risk in patients with coronary stents requiring surgery. Other reasons for stopping or interrupted antiplatelet therapy include patient non-compliance, bleeding complications and financial pressure.

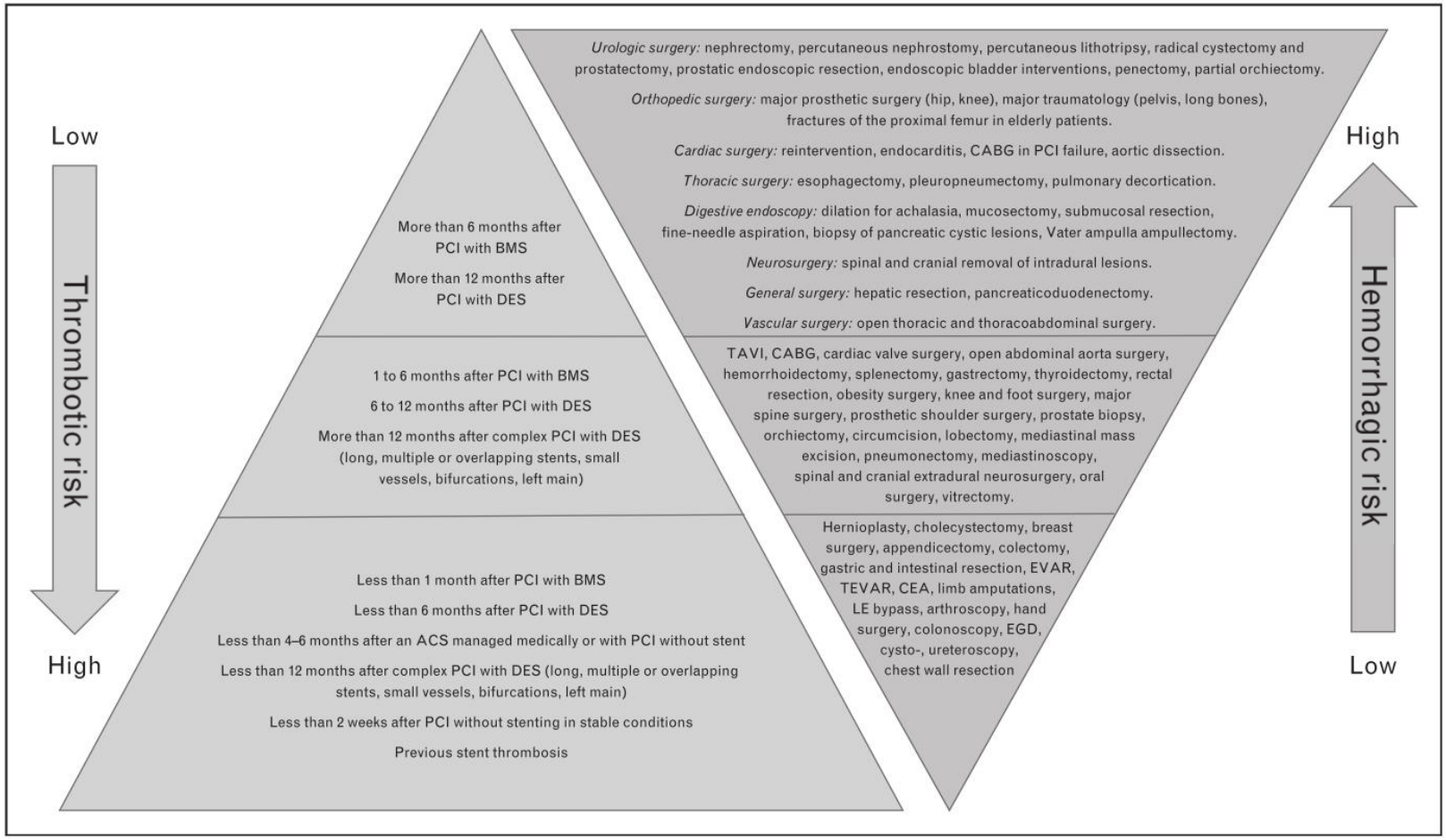


Figure 1-8: Balance between thrombotic and haemorrhagic risk in patients with coronary stents requiring surgery.

(Permissions obtained from Wolters Kluwer Health, License Number 3742551222999)(Franchi, et al., 2014)

1.9 Metabolomics profiling in cardiovascular diseases

Despite the advancements in treatment of cardiovascular diseases, the pathophysiology of these diseases is very complex. Thus, if we are able to predict who is at risk for an acute cardiovascular event, it would be beneficial as we can prevent this event. One of the development and technology that facilitate the discovery of biomarkers is metabolomics technique. Metabolomics has been used as a research tool to understand pathophysiology and mechanisms involve in pathways of interest and also as detection and identifying new biomarkers in diseases of interest.

Metabolomics involves a technology using the methods of separation and detection complex of small molecules that characterize biochemical pathways of interest. The small molecules can be either from intermediate or final products of metabolism within a biological sample. The small molecules include carbohydrates, peptide, lipids, amino acids and drug metabolites (Martinez-Pinna, et al., 2010). In sets of biological samples, the patterns and changes of these metabolites can be measured. Thus, these detected and significant metabolites are useful for identifying new biomarkers. In patients who are taking DAPT and at risk of developing cardiovascular event after stopping DAPT, identification of significant biomarker could reveal pathophysiologic mechanisms behind this.

1.10 Summary of literature review and rationale for the present study

Cardiovascular disease is a leading cause of death and disability worldwide. Effective treatments exist, but there are several outstanding questions. We do not know whether a person who has a stroke or TIA and is already taking aspirin be prescribed the same or an increased dose of aspirin after the stroke. We do not know whether to change their antiplatelet regimen for this group of patient. Furthermore, we are not sure the risk associated with stopping or interrupting antiplatelet early after stroke. At the same time, we want to know more about the risk associated when changing DAPT to single and we do not know if there

are any metabolite changes associated when stopping DAPT. Thus, the aims and objectives listed below will be used as knowledge gaps that need to be filled.

1.11 Aim and objectives of the thesis

1.11.1 Aim

To assess certain aspects of clinical equipoise when using antiplatelet in routine practice.

1.11.2 Objectives

1. To evaluate whether, in patients who suffer ischaemic stroke whilst taking antiplatelet, a change of antiplatelet regimen is associated with a lower risk of recurrence than continuing on the same antiplatelet drug.

2. To evaluate whether, in patients who have suffered ischaemic stroke, early initiation of antiplatelet is associated with better outcomes than later initiation.

3. To evaluate whether, in patients who have suffered ischaemic stroke, early cessation, stopped or interrupted of antiplatelet is associated with an increased risk of cardiovascular event than persistent antiplatelet regimen.

4. To assess the incidence and predictors of cardiovascular event after discontinuation of DAPT in patients who have suffered ACS.

5. To determine whether, in patients who have suffered ACS, there are metabolite changes that are affected after stopping of DAPT.

2 Materials and Methods

2.1 Introduction

Several studies have been conducted in order to achieve the aims and objectives listed in the previous chapter. These include data analyses using multiple data sources and a prospective experimental study. The studies included patients with CHD and cerebrovascular disease.

Three studies were performed using data from the Virtual International Stroke Trials Archive (VISTA) and one study was performed using data contained in the National Health Service Greater Glasgow and Clyde (NHSGGC) Safe Haven. The prospective study included patients with recent acute coronary syndrome who were taking DAPT.

2.2 Data source

2.2.1 VISTA database

VISTA is a collaborative registry that collates and provides access to completed acute stroke trial data and it was established in 2001. VISTA data are stored at the Robertson Centre for Biostatistics, University of Glasgow, Glasgow, UK. Data in VISTA are anonymized in relation to patients' and trials' identity, as the majority of the informed consent and institutional review board approvals that have been gathered restrict storage and transmission to anonymized data (Ali, et al., 2007).

VISTA contains patients' demographic data such as age, sex and ethnicity; smoking history and medical histories including diabetes, hypertension, prior stroke, transient ischaemic attack and myocardial infarction. The date of stroke onset, race, height, weight, and baseline blood pressure are also available for selected trials. The majority of data in VISTA includes acute trials, thus, only short-term outcomes (between 21 to 180 days) are available within VISTA. The outcome measures available include 90-day Scandinavian Stroke Scale; Barthel Index; modified Rankin Scale (mRS); National Institutes of Health Stroke Scale (NIHSS); and Orgogozo, Mathews, and European Stroke Scales. Medication

details are also available within VISTA. Adverse events (AE) and some laboratory measurements are available from certain trials. While the main aim of VISTA is to facilitate the planning of future randomized clinical trials, the VISTA database does not permit further testing of any trial data that will test treatment effects in any individual trial (Ali, et al., 2007; Ali, 2008).

Data extraction

For the purpose of my research, VISTA data were obtained from VISTA Acute which contains trials that were conducted between 1998 and 2008. A proposal to acquire VISTA data was sent and data on patient's baseline characteristics; drug history which include prior and current medications; outcomes which include recurrent stroke, cardiovascular event, mRS at 90 days and NIHSS at 90 days; and AEs which include minor and major bleeding and ICH were requested. Based on these data requirements, I was given accessed to an anonymised dataset of 10,304 patients from VISTA. The use of the anonymised dataset for research studies is approved by the VISTA-Acute Steering Committee (letter dated 7 Feb 2013) (**Appendix 1**).

Data cleaning and coding

The anonymised dataset obtained from VISTA were given in five different datasets. There were datasets for baseline and clinical characteristics, prior medications, current medications, AEs and serious adverse events (SAE). The baseline and clinical characteristics dataset was straightforward. However, the rest of datasets are needed to be cleaning and coding before it can be used.

In VISTA, the majority of medications were coded using the World Health Organization's Anatomical Therapeutic Chemical (ATC) classifications. In this classification system, drugs are divided according to the organ or system on which they act and according to their chemical, pharmacological and therapeutic properties (World Health Organization (WHO) Collaborating Centre, 2003). For medications without ATC codes, their ATC codes were coded manually. Medications that used brand names were changed manually to their generic names, for example, Trombyl which is also known as aspirin. The coding for both prior and current medications were done separately. Then, each dataset was sorted by patient identification number and medication's generic

name. The data on prior antiplatelet treatment with ATC code: B01AC were extracted into a new dataset (prior antiplatelet dataset). SAS software was used to transpose antiplatelet generic name, start day and end day of each antiplatelet agent according to patient identification number. This step was repeated for the current medication dataset. These datasets i.e. prior antiplatelet and current antiplatelet were then merged with the baseline and clinical characteristics dataset based on patient identification number.

Whereas for AE and SAE dataset, specific keywords were used and searched. For recurrent stroke, the keywords used include infarct or ischaemia or ischaemic or stroke or cerebrovascular accident (CVA). The terms used for the extraction can be found in **Appendix 2**. For ACS, the terms used were unstable angina or acute coronary syndrome or myocardial infarction. For transient ischaemic attack, the term used as it is. For bleedings, the terms used to extract this information is listed in **Appendix 3**. Afterwards, each dataset was sorted by patient identifier and recurrent stroke event. SAS software was used to transpose recurrent stroke, the AE start day and end day according to patient identification number. These datasets then merged with the baseline and clinical characteristics dataset by patient identification number. The process was repeated for the rest of AE, i.e. ACS, TIA, intracranial haemorrhage (ICH) and extracranial haemorrhage (ECH).

Patient selection in VISTA

For VISTA analysis, all patients with missing baseline characteristics and antiplatelet information were excluded especially patients with missing information on initiation day of antiplatelet therapy. Furthermore, patients with concurrent use of vitamin K antagonist such as warfarin were excluded as it may influence clinical (Abdul-Rahim, et al., 2014) and safety (Shireman, et al., 2004) outcomes in acute ischaemic stroke patients. A flow diagram showing the selection of final data from VISTA is presented in figure 2.1. The inclusion and exclusion criteria for each study using VISTA dataset are described in their individual section in Chapter 3, 4 and 5.

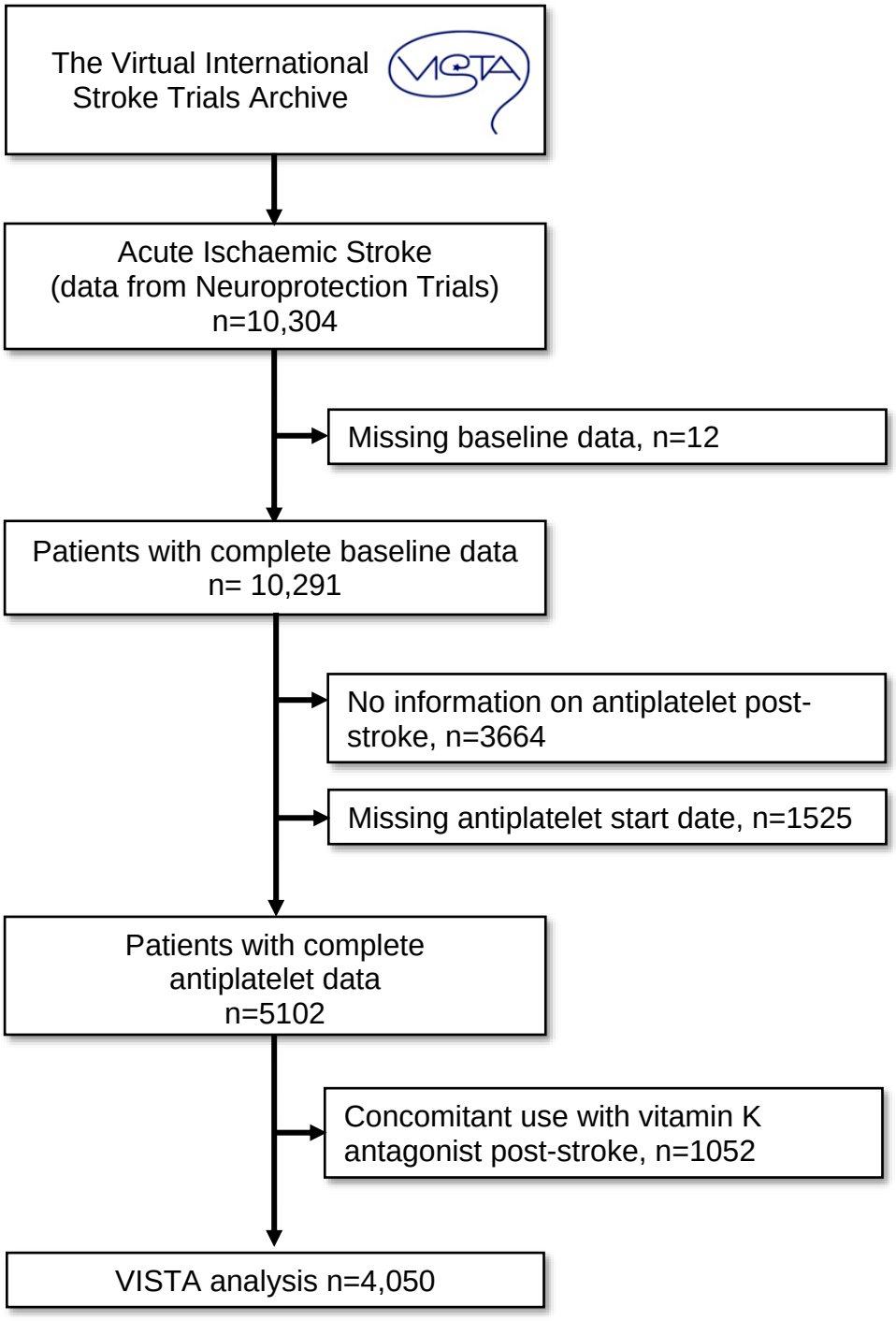


Figure 2-1: Flow chart for selection of ischaemic stroke patients from VISTA.

2.2.2 NHS Greater Glasgow and Clyde Safe Haven

NHS Scotland has set up regional Safe Havens located within Aberdeen, Dundee, Edinburgh and Glasgow, for delivering research excellence and the need for rapid access to high quality health data for research purposes (NHS Research Scotland, 2016). NHSGGC and the Robertson Centre for Biostatistics develop a Safe Haven and provide access to local healthcare data in the West of Scotland. In the Safe Havens, they consist of many administrative datasets that are useful for research purposes such as Scottish Morbidity Record (SMR01), General Register Office for Scotland (GROS) death registration and Prescribing Information System. The SMR01 collects information on all admissions to acute care hospitals in Scotland, including the date of the admission, the type of admission, the disease codes for the current admission and admissions for surgical procedures. The GROS death registration collects information on all deaths that occur in Scotland including the date of death. The PIS database records the date dispensed and the number of days supplied for each dispensed medication.

Data extraction

For the purpose of my research, the SMR01 database was used to search all admission for ACS that occurred in the West of Scotland. A proposal to acquire this dataset was sent to NHSGGC Safe Haven team and data on patient's baseline characteristics; prescribing history; diagnosis of ACS which include unstable angina, STEMI and NSTEMI; stroke; death and major bleeding complications which include subarachnoid, intracranial, intraocular and retroperitoneal haemorrhage were requested. Based on these data requirements, I was given accessed to an anonymised dataset of 7232 ACS patients hospitalised from between 2008 and 2013. The use of the anonymised dataset for research studies is approved by the Local Privacy Advisory Committee (LPAC) group, NHSGGC (GSH/13/CA/005) (**Appendix 4**). The details of patient extraction are shown in Figure 2-2.

Data cleaning and coding

The anonymised dataset obtained from NHSGGC were given in three different datasets. There were datasets for demographic data, admissions for ACS and

antiplatelet prescribing history. Demographic dataset contains information on patient date of birth, date of death and gender. Demographic dataset was straightforward. Similarly, as VISTA data, the rest of datasets are needed to be cleaning and coding before it can be used.

The admission dataset contains information on date of admission, diagnosis for the current admission and admissions for surgical procedures. This dataset was also used to search for recurrent ACS, ischaemic stroke, TIA, heart failure, cardiac death and major bleeding following the first admission of ACS. The ACS, ischaemic stroke, heart failure, cardiac death (**Appendix 5**) and major bleeding (**Appendix 6**) were coded based using the International Classification of Diseases, Tenth Revision (ICD-10) codes. There were patients with more than one admission, thus each event was reviewed with their date of admission manually. Then, this dataset was sorted by patient identifier. SAS software was used to transpose recurrent stroke, AE start day and end day according to patient identification number. These datasets then merged with the demographic dataset based on patient identification number.

Antiplatelet prescribing dataset in NHS Scotland data were coded using the BNF (British National Formulary). The BNF Code contains 15-digit code, where the first seven digits are according to the drug classes in the BNF and the last 8 digits characterize the medicinal product, form, strength and the link to the generic equivalent product (NHS National Services Scotland). In this dataset, it also includes antiplatelet strength dispensed to the patient, dispensed date and dispensed quantity. Dual antiplatelet therapy regimen (Figure 2-2) and duration of antiplatelet prescribed for each patient were searched and calculated manually using the data provided. Then, this dataset was sorted by patient identification number and antiplatelet regimen. SAS software was used to transpose antiplatelet regimen, start day and end day of each medication according to patient identification number. This dataset then merged with the demographic dataset according to patient identification number.

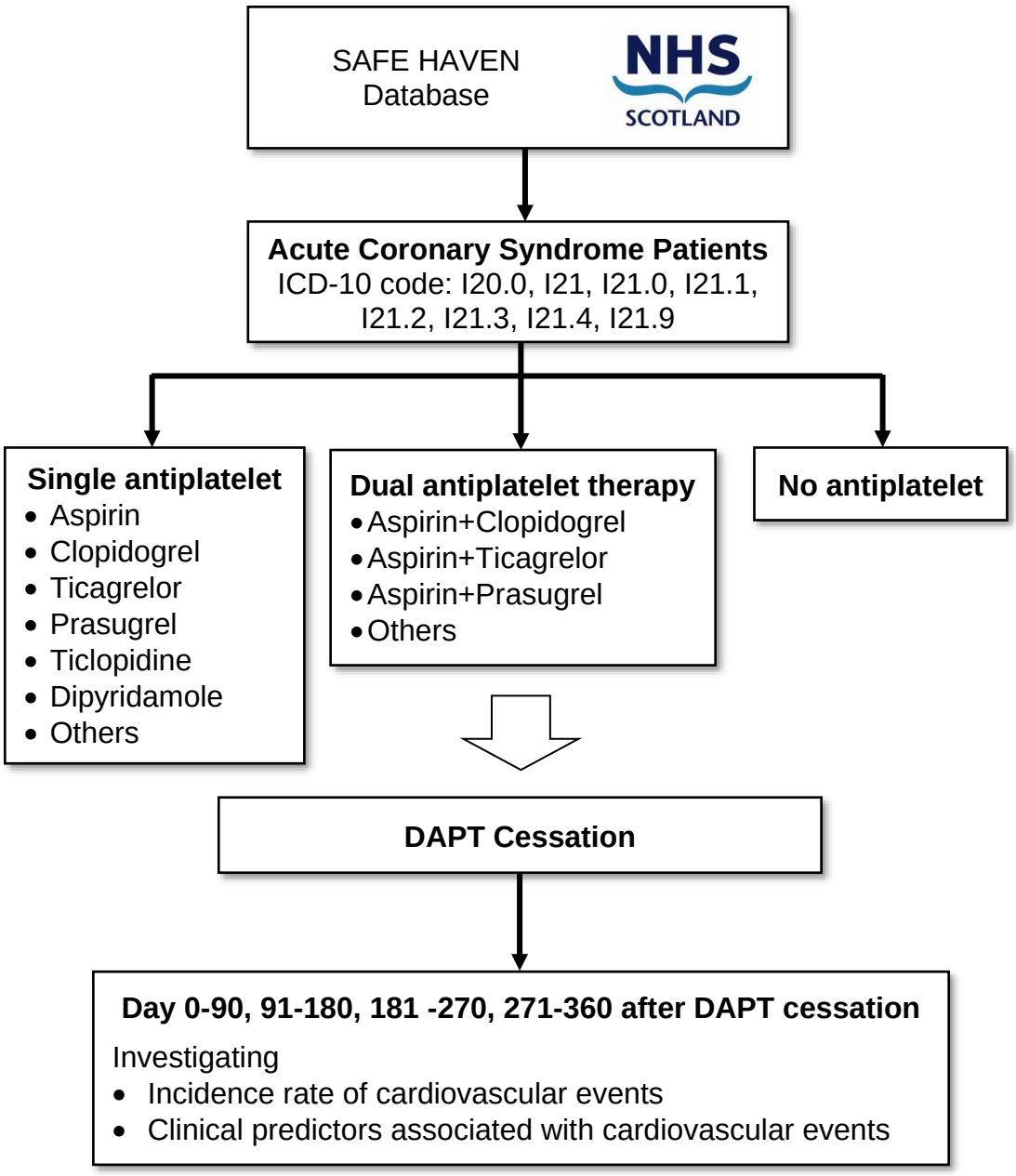


Figure 2-2: Flow chart for selection of acute coronary syndrome from NHS Scotland National Safe Haven.

ICD=International Classification of Diseases, DAPT=dual antiplatelet therapy.

2.3 Outcome measures

2.3.1 VISTA analysis

There are several outcomes measured in VISTA analysis. Because of VISTA characteristics, short-term outcomes within 90 days after acute ischaemic stroke were used. The primary efficacy outcome was a recurrent ischaemic stroke at 90 days. This information about recurrence ischaemic stroke was extracted in all patients from AE and SAE reports.

The primary safety outcome was a bleeding complication. This information was extracted from AE and SAE datasets. Bleeding complications were divided into two different categories i.e. ICH and ECH. Intracranial haemorrhage was defined as any ICH and haemorrhagic transformation 1 and 2 of cerebral infarction were excluded. Whereas, ECH was defined as gastrointestinal (GI) and non-GI bleeding. Gastrointestinal bleeding was defined as patients with overt GI bleeding, whereas non-GI bleeding with no evidence of overt GI bleeding. The list of key terms used to identify ICH and ECH is listed in **Appendix 3**.

Another outcome measures used in VISTA analysis is modified Rankin Scale (mRS). It is one of the common outcome measures that were used in stroke research (Quinn, et al., 2009). The mRS is a scale used to assess degree of disability or dependence in daily activities after stroke. It is ordinal hierarchical scale ranging from 0 to 6 which assess functional status of patients. This information is available within VISTA, thus it was used to measure functional outcome at day 90 after acute ischaemic stroke. The mRS scores as described in Table 2-3.

Table 2-1: Scores on a modified Rankin scale (Saver, 2007).

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to perform all usual duties and activities
2	Slight disability; unable to perform all previous activities, but able to take care of self without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent, and requiring constant nursing care and attention
6	Dead

2.3.2 NHS Scotland National Safe Haven analysis

For NHS SNSH analysis, cardiovascular events occurred following ACS and after DAPT cessation were measured. Cardiovascular events include recurrent ACS, ischaemic stroke and death. Further description of this study will be described more in Chapter 6.

2.4 Experimental study

The study participants, ethics approval, inclusion and exclusion criteria, and study methods for the prospective study are described in Chapter 7.

2.5 Statistical analysis

Statistical advice was sought from Stroke Trials statistician Dr Rachael MacIsaac at Acute Stroke Unit, Institute Cardiovascular and Medical Sciences, University

of Glasgow. The statistical methods used for each study are described in the methodology section of each chapter.

2.6 Statistical software for analyses

Statistical analyses were performed with IBM SPSS Statistics version 21.0 (IBM Corp, 2012).

3 Antiplatelet therapy following ischaemic stroke – Continue or change pre-existing therapy?

3.1 Introduction

Antiplatelet therapy is recommended for patients with ischaemic stroke or transient ischaemic attack (TIA) due to atherosclerotic disease (Hacke, et al., 2008b; Furie, et al., 2011; Lansberg, et al., 2012; Jauch, et al., 2013). Antiplatelet drugs are prescribed to prevent further ischaemic stroke, minimize the risk of early death and to improve long-term outcome (Gent, et al., 1996). Use of aspirin leads to fewer deaths (nine per 1000 treated) and more patients with a good functional outcome (seven per 1000 treated) at 30 days after ischaemic stroke compared to no aspirin use (Sandercock, et al., 2008) and in the longer term antiplatelet drugs reduce the risk of recurrent cardiovascular events and prevents approximately 1/5 of recurrent strokes (Baigent, et al., 2002; Baigent, et al., 2009).

Guidelines recommend that antiplatelet regimens choices are individualised based on numerous factors including patient risk factor profiles, cost, tolerability and relative effectiveness (Kernan, et al., 2014). One potentially important factor is pre-existing anti-platelet therapy. In the recently published CHANCE study, at least 20% of patients had a history of previous stroke and 11% of patients had their index stroke whilst taking antiplatelet therapy (Wang, et al., 2013). In North Dublin Stroke study, 34.8% patients in their sample were taking antiplatelet therapy before first-ever stroke (Kelly, et al., 2012).

Guidelines acknowledge that in patients who experience a stroke whilst taking antiplatelet therapy, there are no clinical trials to show whether switching antiplatelet agents decreases the risk of subsequent events (Furie, et al., 2011). There are reasons why switching regimen may be advantageous. Failure of antiplatelet treatment could be due to disease severity (no drug will be 100% effective), lack of adherence or resistance to the effect of the antiplatelet

drug. Resistance to clopidogrel may be present in 10 to 25% of patients and associated with an increased risk of cardiovascular events (Muller, et al., 2003; Matetzky, et al., 2004; Serebruany, et al., 2005b; Wang, et al., 2010). It is possible resistant patients should be switched to another class of antiplatelet. In this study, we compared the rate of recurrent stroke and bleeding complications after ischaemic stroke in patients who were already taking antiplatelet drugs according to whether they continued on the same drugs or changed regimen.

3.2 Methods

3.2.1 Study setting and study population

General details of the study setting and study population are described in section 2.2.1. Briefly, the data used are contained in the Virtual International Stroke Trials Archive (VISTA) database. The VISTA data are anonymous to included patients and clinical trials. We included participants who had suffered ischaemic stroke whilst taking antiplatelet drugs (for at least 7 days prior to stroke) and who were recommenced on antiplatelet therapy within 90 days after ischaemic stroke.

3.2.2 Definition of antiplatelet Exposure

Data on antiplatelet treatments were based on platelet aggregation inhibitors (which includes derivatives of salicylic acid, thienopyridine and dipyridamole). Patients were divided into two different groups; a change or continue group. The change group comprised patients who changed to a new antiplatelet regimen after the index ischaemic stroke. The continue group comprised patients who continued the same antiplatelet therapy after the index ischaemic stroke. Further details will be explained using Figure 3-1.

Schematic diagram for antiplatelet exposure and recurrent events for four subjects is shown in Figure 3-1. Antiplatelet regimen before the stroke represented by continuous line. For those who continued with similar antiplatelet regimen following an acute stroke, the continuous line continued

until an event occurred, represented by black dots (subject A) or until the study end, represented by unfilled circle at the end of individual's line segment (subject B). Subject A and subject B were categorized as continue group. On the other hand, for patients who changed to a new antiplatelet regimen, the continuous line was changed to the dashed line (subject C and subject D). Subject C and subject D were categorized as change group. Among those subjects, two had an event (subject A and subject C). Two of the subjects were censored (subject B and subject D) at the end of the study, at 90 days.

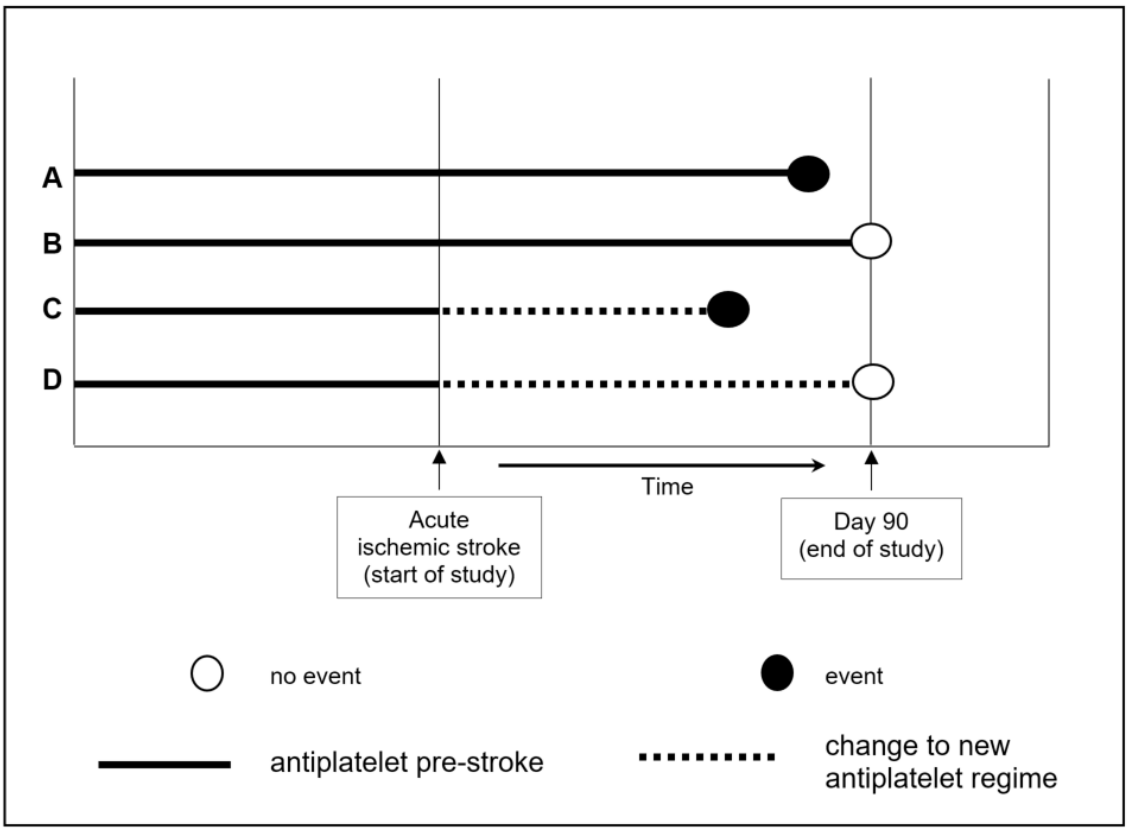


Figure 3-1: Schematic depiction of antiplatelet exposure (represented by continuous or dashed line), recurrent event (represented by black dots) and censoring data (represented by unfilled circle) for four patients, with a time dependent covariate.

3.2.3 Outcome measures

The primary outcome was the occurrence of recurrent ischaemic stroke and the secondary outcome was bleeding complications within 90 days post-stroke. The modified Rankin Scale (mRS) at 90 days was used to measure functional outcomes. The information about recurrent ischaemic stroke and bleeding

complications in all patients were extracted from AE and SAE reports. A recurrent ischaemic stroke was defined as a clear statement of a new adverse event including terms ischaemic stroke, cerebral infarction or cerebrovascular accident. Other events such as worsening of initial stroke symptoms or oedema were not included.

For bleeding complication, the event was identified using these key terms: hematoma, haemorrhage, bleeding, blood or melaena. Bleeding complications were categorized into two i.e. intracranial haemorrhage (ICH) and extracranial haemorrhage (ECH). Intracranial haemorrhage was defined as any ICH but excluding haemorrhagic transformation infarction 1 and 2. Exclusion of haemorrhagic transformation was done by identifying these key terms: haemorrhagic conversion (1 and 2) or haemorrhagic transformation (1 and 2). Whereas, ECH was defined as any bleeding episode from another source and this includes gastrointestinal (GI) and non-GI bleeding. The information on data extraction from the VISTA has been described in Chapter 2 (section 2.2.1 and section 2.3.1).

3.2.4 Statistical analysis

Descriptive statistics were recorded for recent stroke patients, comparing those who changed antiplatelet regimen versus those who continued the same antiplatelet regimen post-stroke. For categorical variables such as demographics and medical history data, they were summarised using frequencies and proportions and were compared using the Chi-square test or Fisher's exact test, where appropriate. Whereas for continuous variables, they were summarised using mean [standard deviation (SD)] or median [interquartile range (IQR)] and were compared using Student t-test or non-parametric Mann-Whitney test.

Our outcome measures were the occurrence of recurrent stroke, ICH, ECH by 90 days post-stroke and the ordinal shift of the mRS at day 90 using the full scale. We calculated the odds ratio (OR) and corresponding 95% confidence intervals (95% CI) using binary logistic regression for dichotomized outcome and ordinal logistic regression for mRS distribution. Adjustments were made for age

and baseline NIHSS. Age and baseline severity are the two most powerful prognostic factors for stroke and are usually included in outcome distribution analyses (Lees, et al., 2006; Konig, et al., 2008; Bath, et al., 2012).

We also assessed the effect of changed or continued antiplatelet regimen on outcome measures in subgroups: clinical condition (atrial fibrillation or not, prior stroke or not and treated with intravenous thrombolysis or not). We conducted subgroup analyses in these populations as their stroke risk recurrence (Burn, et al., 1994; Penado, et al., 2003) and neurological recovery (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995) are different. Similarly, the same method was repeated by comparing patients who were changed from aspirin to clopidogrel to those who continued aspirin with aspirin post-stroke.

A p-value <0.05 was used to define statistical significance. All analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp, 2012).

3.3 Results

3.3.1 Patients sample

Complete data on antiplatelet therapy were available for analysis of the outcome measures in 4050 patients. (See Figure 2-1 for information on the completeness of VISTA data).

Of 4050 ischaemic stroke patients, 2921 were excluded as they have no prior use of antiplatelet therapy. Demographic and patient characteristics for the remaining 1129 stroke patients who were included cohort (Figure 3-2) are shown in Table 3-1. Of the 1129 patients, 538 (47.7%) were changed to a new antiplatelet drug post-stroke, whereas 591 (52.3%) were not. The change group comprised a higher percentage of men, a lower rate of atrial fibrillation, a lower NIHSS score, and a higher percentage of previous stroke.

Table 3-2 shows proportion of antiplatelet used based on prior antiplatelet. The majority of subjects in this study were taking aspirin prior to stroke and

approximately half of them continued on aspirin after stroke. Likewise, about half of subjects were taking aspirin after stroke. Nearly half of those subjects in change group were changed to clopidogrel and about half of those subjects were taking aspirin previously.

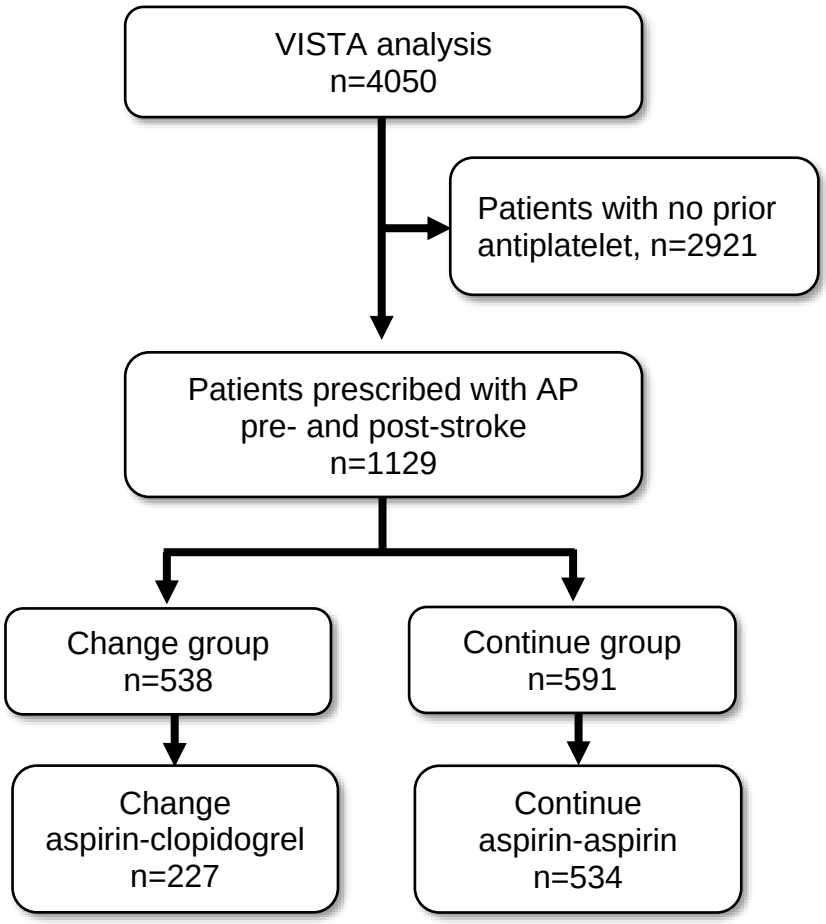


Figure 3-2: Selection of eligible patients includes in data analyses.

Table 3-1: Baseline characteristics

Characteristics	Change (n=538)	Continue (n=591)	p-value
Age, years*	70.91 (11.25)	72.08 (10.49)	0.091
Male	332 (61.7)	329 (55.7)	0.040
Caucasian	469/527 (89.0)	531/571 (93.0)	0.020
Current Smoker	172/524 (32.8)	145/471 (30.8)	0.491
Baseline NIHSS†	13 (9-17)	14 (9-18)	0.036
Medical history			
Hypertension‡	444/537 (82.7)	448/590 (75.9)	0.005
Diabetes‡	153/538 (28.4)	155/590 (26.3)	0.414
AF‡	79/537 (14.7)	135/590 (22.9)	0.000
Heart failure‡	48/513 (9.4)	51/548 (9.3)	0.978
IHD‡	229/525 (43.6)	204/471 (43.3)	0.922
Prior TIA‡	68/490 (13.9)	67/554 (12.1)	0.391
Prior stroke‡	190/527 (36.1)	133/568 (23.4)	0.000
Thrombolysed, rt-PA	234 (43.5)	174 (29.4)	0.000
AP duration post-stroke			
≤ 30 days	109 (20.3)	156 (26.4)	0.041
31- 60 days	36 (6.7)	31 (5.2)	
61- 90 days	393 (73.0)	404 (68.4)	

All values are reported as no. (%) unless otherwise noted. *Values are reported as mean (SD); †median (IQR); ‡missing some data at baseline. AF=atrial fibrillation, AP=antiplatelet, IHD= ischaemic heart disease, NIHSS=National Institutes of Health Stroke Scale, rt-PA=recombinant tissue plasminogen activator. TIA=transient ischaemic attack, SD=standard deviation, IQR=interquartile range.

Table 3-2: Proportion of antiplatelet pattern pre- and post-stroke

Antiplatelet Pre-stroke	Antiplatelet post-stroke						Total
	Aspirin	Aspirin+ Clopidogrel	Aspirin+ Dipyridamole	Clopidogrel	Dipyridamole	Others	
Aspirin	534 (82.3)	27 (62.8)	64 (73.6)	227 (85.7)	13 (86.7)	35 (50.0)	900 (79.7)
Aspirin+clopidogrel	19 (2.9)	4 (9.3)	5 (5.7)	6 (2.3)	0 (0.0)	0 (0.0)	34 (3.0)
Aspirin+dipyridamole	11 (1.7)	2 (4.7)	10 (11.5)	3 (1.1)	0 (0.0)	2 (2.9)	28 (2.5)
Clopidogrel	46 (7.1)	10 (23.3)	3 (3.4)	21 (7.9)	0 (0.0)	0 (0.0)	80 (7.1)
Dipyridamole	6 (0.9)	0 (0.0)	2 (2.3)	1 (0.4)	1 (6.7)	1 (1.4)	11 (1.0)
Others	33 (5.1)	0 (0.0)	3 (3.4)	7 (2.6)	1 (6.7)	32 (45.7)	76 (6.7)
Total	649 (100)	43 (100)	87 (100)	265 (100)	15 (100)	70 (100)	1129 (100)

All values are reported as no. (%) unless otherwise noted.

3.3.2 Clinical outcomes

There was no difference in recurrent stroke rate between groups. Recurrent stroke occurred in 22 patients (4.1%) in the change group, as compared with 25 patients (4.3%) in the continue group (adjusted OR, 0.97; 95% CI, 0.54-1.75; p=0.929) (Table 3-3).

The numbers of patients with ICH were 13 (2.4%) in the change group and 15 (2.6%) in the continue group. We found no significant difference between the two groups (adjusted OR, 1.02; 95% CI, 0.48-2.18; p=0.955) (Table 3-3). Similarly, there was no significant difference in the rate of ECH event between the change (25 [4.7%]) and continue groups (17 [2.9%]) (adjusted OR, 1.82; 95% CI, 0.96-3.43; p=0.065).

The distribution of mRS at 90 days for change versus continue group is shown in Figure 3-3(a). We found that change to a new antiplatelet post-stroke was associated with more favourable functional outcome across a full scale mRS at 90 days after adjustments for age and baseline NIHSS (adjusted OR, 1.48; 95% CI, 1.19-1.86; p=0.0006), compared with continue group. Additional analyses adjusted for age, baseline NIHSS, hypertension, AF, previous TIA, previous stroke, and treated with rt-PA are shown and illustrated in **Appendix 7**, showing a significant increase risk of ECH in change group compared with continue group (adjusted OR, 2.03; 95% CI, 1.04-3.98; p=0.039).

Table 3-3: Clinical outcomes at 90 days (adjusted for age and baseline NIHSS)

Outcomes	Change n=538	Continue n=591	Adjusted OR (95% CI)	p-value
Recurrent stroke	22/532 (4.1)	25/588 (4.3)	0.97 (0.54-1.75)	0.929
ICH	13/532 (2.4)	15/581 (2.6)	1.02 (0.48-2.18)	0.955
ECH	25/527 (4.7)	17/584 (2.9)	1.82 (0.96-3.43)	0.065

All values are reported as no. (%) unless otherwise noted. CI=confidence interval, ICH=intracranial haemorrhage, ECH=extracranial haemorrhage, OR=odds ratio.

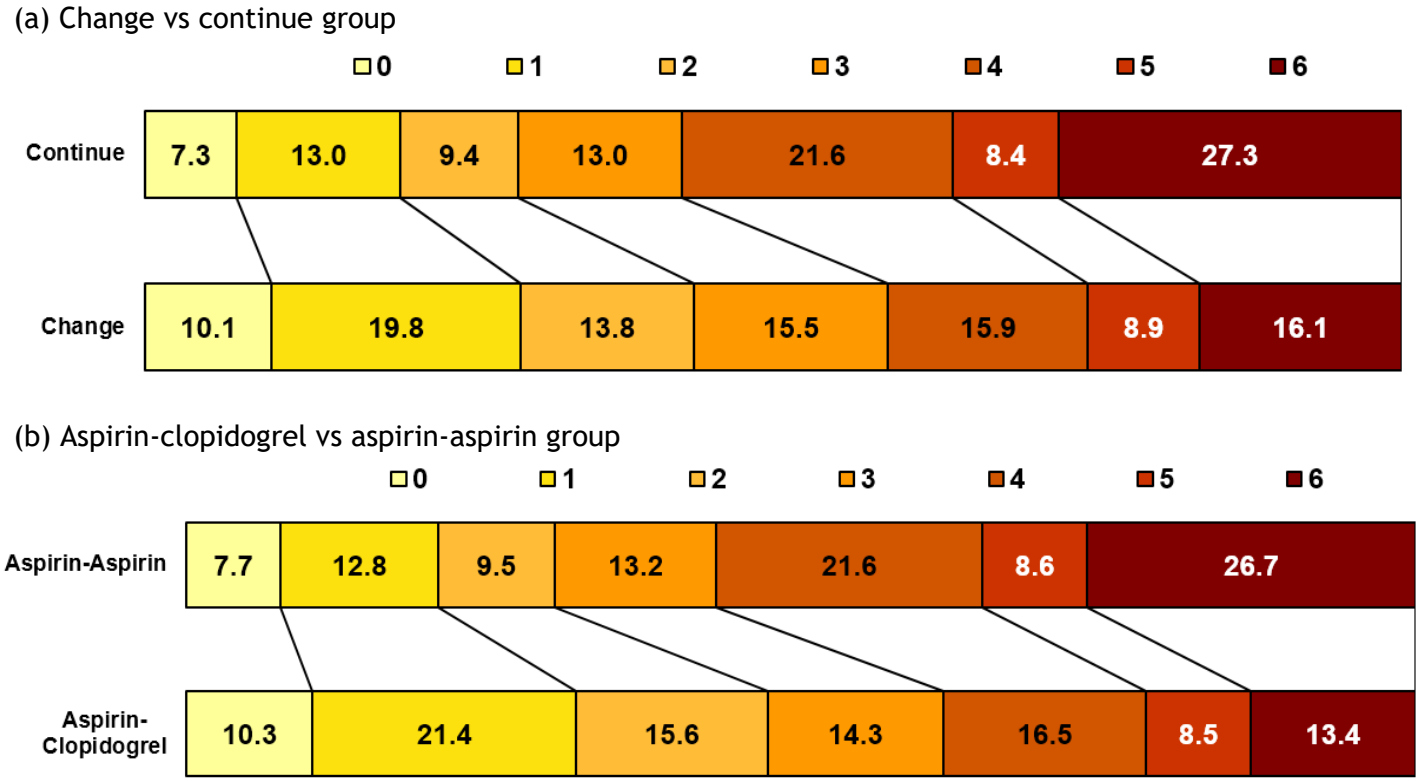


Figure 3-3: Distribution of mRS outcome at day 90 in patients suffering ischaemic stroke.

Diagram showing association of functional outcome at day 90 between (a) change and continue group and (b) aspirin-clopidogrel and aspirin-aspirin group. Values provided in each box denote the percentage of patients belonging to a specific treatment category (change or continue) and representing the mRS score corresponding to the box.

3.3.3 Subgroup analysis

There is a significant difference in the prevalence of atrial fibrillation between the two groups being higher in the continue group, and the prevalence of prior stroke and thrombolysis treated patients being higher in the change group (Table 3-1). Analyses of these subgroups are presented in Figure 3-4. However, we did not find a significant influence on the occurrence of recurrent stroke, ICH and ECH events between the change and continue group in these populations.

	Change n=538	Continue n=591	Adjusted OR (95% CI)	Adjusted OR (95% CI)	p-value
Recurrent ischaemic stroke					
AF	3/78 (3.8)	5/132 (3.8)		1.05 (0.24-4.70)	0.946
No AF	19/453 (4.2)	20/455 (4.4)		0.96 (0.51-1.83)	0.906
Prior stroke	4/187 (2.1)	7/131 (5.3)		0.39 (0.11-1.36)	0.140
No prior stroke	18/334 (5.4)	18/434 (4.1)		1.32 (0.68-2.60)	0.414
Thrombolysed	8/231 (3.5)	8/171 (4.7)		0.75 (0.27-2.05)	0.568
Non-thrombolysed	14/301 (4.7)	17/417 (4.1)		1.14 (0.55-2.36)	0.723
Intracranial haemorrhage					
AF	3/78 (3.8)	3/134 (2.2)		2.26 (0.42-12.32)	0.346
No AF	10/453 (2.2)	12/446 (2.7)		0.84 (0.36-1.98)	0.698
Prior stroke	5/186 (2.7)	4/132 (3.0)		1.01 (0.26-3.92)	0.985
No prior stroke	8/335 (2.4)	9/428 (2.1)		1.30 (0.49-3.48)	0.596
Thrombolysed	9/229 (3.9)	5/166 (3.0)		1.44 (0.47-4.43)	0.522
Non-thrombolysed	4/303 (1.3)	10/415 (2.4)		0.59 (0.18-1.91)	0.377
Extracranial haemorrhage					
AF	5/76 (6.6)	6/134 (4.5)		1.66 (0.47-5.84)	0.433
No AF	20/450 (4.4)	11/449 (2.4)		1.93 (0.91-4.09)	0.087
Prior stroke	10/184 (5.4)	6/131 (4.6)		1.31 (0.46-3.75)	0.614
No prior stroke	15/333 (4.5)	11/431 (2.6)		1.98 (0.89-4.41)	0.096
Thrombolysed	8/227 (3.5)	3/171 (1.8)		2.50 (0.63-9.83)	0.191
Non-thrombolysed	17/300 (5.7)	14/413 (3.4)		1.82 (0.87-3.78)	0.111

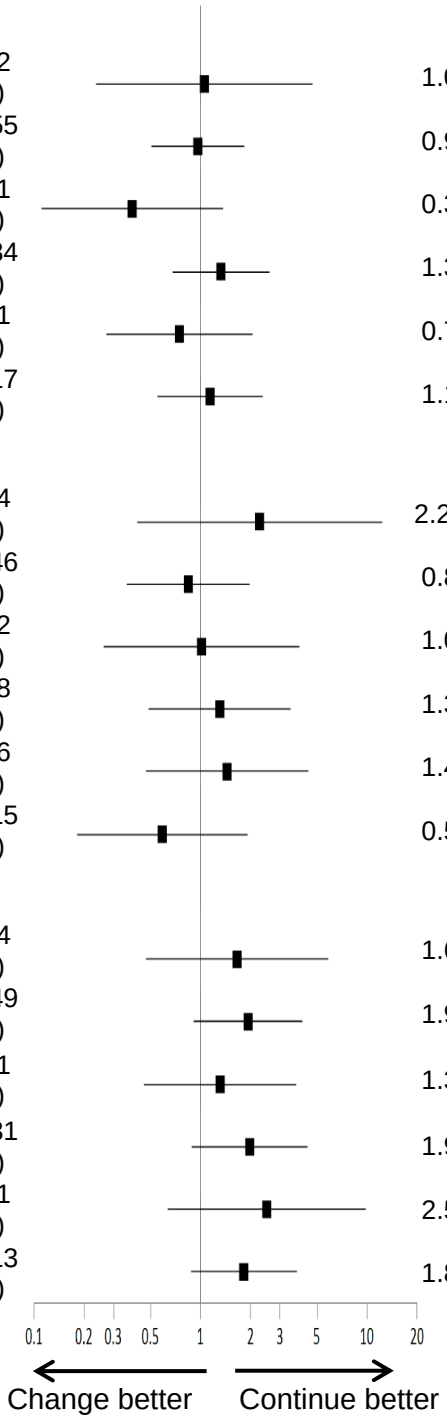


Figure 3-4: Clinical outcomes at 90 days (adjusted for age and baseline NIHSS) by subgroup

All values are reported as no. (%) unless otherwise noted. AF=atrial fibrillation, OR=odds ratio, CI=confidence interval.

3.3.4 Additional analyses

We formed another cohort from 761 ischaemic stroke patients, of whom 227 (29.8%) were changed from aspirin to clopidogrel and 534 (70.2%) were remained with aspirin post-stroke. A flow diagram showing the selection of this cohort is presented in Figure 3-2. Detailed baseline characteristics are given in Table 3-4.

The effect of aspirin-clopidogrel group versus aspirin-aspirin group on the rates of the recurrent stroke, ICH and ECH were comparable between these two groups. Similarly, in subgroup analysis, there was no significant different on the occurrence of recurrence stroke, ICH and ECH across subgroups. Adjusted ORs (95% CI) are provided in Figure 3-5. We also found that change from aspirin to clopidogrel post-stroke was associated with more favourable functional outcome across full scale mRS at 90 days after adjustments for age and baseline NIHSS (adjusted OR, 1.59; 95% CI, 1.19-2.13; p=0.0018), compared with aspirin-aspirin group (Figure 3-3(b)).

Table 3-4: Baseline characteristics

Characteristics	Aspirin-Clopidogrel n=227	Aspirin-Aspirin n=534	p-value
Age, years*	71.56 (11.213)	72.17 (10.427)	0.497
Male	138 (60.8)	296 (55.4)	0.172
Caucasian	207/226 (91.6)	478/515 (92.8)	0.562
Current Smoker	82/227 (36.1)	131/429 (30.5)	0.146
Baseline NIHSS†	12 (8-16)	14 (9-19)	0.014
Medical history			
Hypertension‡	195/227 (85.9)	407/534 (76.2)	0.003
Diabetes‡	65/227 (28.6)	141/534 (26.4)	0.526
AF‡	31/227 (13.7)	126/534 (23.6)	0.002
Heart failure‡	17/222 (7.7)	44/493 (8.9)	0.575
IHD‡	98/227 (43.2)	186/429 (43.4)	0.964
Prior TIA‡	22/209 (10.5)	56/506 (11.1)	0.833
Prior stroke‡	67/227 (29.5)	111/513 (21.6)	0.021
Thrombolysed, rt-PA	104 (45.8)	159 (29.8)	0.000
AP duration post-stroke			
≤ 30 days	38 (16.7)	142 (26.6)	0.014
31- 60 days	14 (6.2)	30 (5.6)	
61- 90 days	175 (77.1)	362 (67.8)	

All values are reported as no. (%) unless otherwise noted. *Values are reported as mean (SD); †median (IQR); ‡missing some data at baseline. AF=atrial fibrillation, AP=antiplatelet, IHD=ischaemic heart disease, IQR=interquartile range, NIHSS=National Institutes of Health Stroke Scale, rt-PA=recombinant tissue plasminogen activator, SD=standard deviation, TIA=transient ischaemic attack.

	Aspirin-Clopidogrel n=227	Aspirin-Aspirin n=534	Adjusted OR (95% CI)	p-value
Recurrent stroke				
All	9/226 (4.0)	24/531 (4.5)	0.87 (0.40-1.90)	0.723
AF	1/30 (3.3)	5/123 (4.1)	0.95 (0.10-8.90)	0.966
No AF	8/196 (4.1)	19/408 (4.7)	0.86 (0.37-2.01)	0.730
Prior stroke	2/67 (3.0)	7/109 (6.4)	0.44 (0.09-2.19)	0.316
No prior stroke	7/159 (4.4)	17/401 (4.2)	1.04 (0.42-2.59)	0.929
Thrombolysed	2/104 (1.9)	8/156 (5.1)	0.35 (0.7-1.70)	0.192
Non-thrombolysed	7/122 (5.7)	16/375 (4.3)	1.33 (0.53-3.36)	0.545
Intracranial haemorrhage				
All	3/226 (1.3)	12/524 (2.3)	0.63 (0.18-2.29)	0.487
AF	0/31 (0)	2/125 (1.6)	-	0.998
No AF	3/195 (1.5)	10/399 (2.5)	0.62 (0.17-2.30)	0.474
Prior stroke	2/67 (3.0)	4/110 (3.6)	0.87 (0.15-4.97)	0.878
No prior stroke	1/159 (0.6)	6/395 (1.5)	0.52 (0.06-4.49)	0.550
Thrombolysed	3/103 (2.9)	4/151 (2.6)	1.22 (0.25-6.03)	0.804
Non-thrombolysed	0/123 (0)	8/373 (2.1)	-	0.996
Extracranial haemorrhage				
All	13/222 (5.9)	17/528 (3.2)	2.00 (0.94-4.24)	0.071
AF	2/29 (6.9)	6/125 (4.8)	1.72 (0.31-9.69)	0.538
No AF	11/193 (5.7)	11/403 (2.7)	2.18 (0.92-5.16)	0.075
Prior stroke	5/65 (7.7)	6/110 (5.5)	1.51 (0.44-5.20)	0.518
No prior stroke	8/157 (5.1)	11/398 (2.8)	2.01 (0.78-5.19)	0.151
Thrombolysed	4/101 (4.0)	3/156 (1.9)	2.40 (0.51-11.19)	0.266
Non-thrombolysed	9/121 (7.4)	14/372 (3.8)	2.14 (0.88-5.21)	0.093

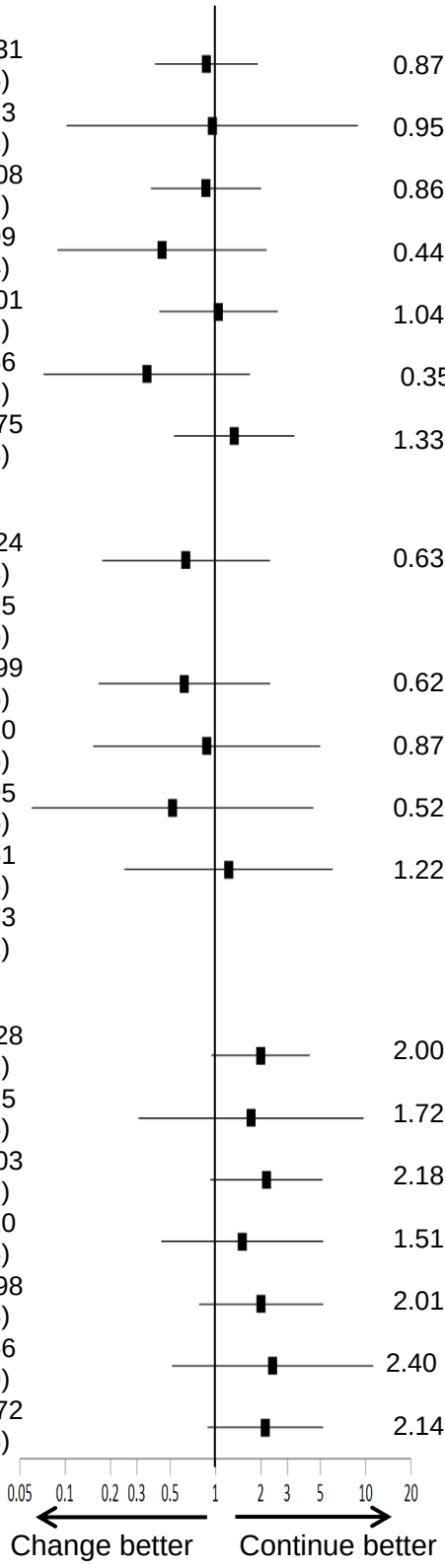


Figure 3-5: Clinical outcomes at 90 days (adjusted for age and baseline NIHSS)

All values are reported as no. (%) unless otherwise noted. AF=atrial fibrillation, OR=odds ratio, CI=confidence interval.

3.4 Discussion

In the present study, we found that subjects who experience stroke while on antiplatelet therapy and who switch to a new class of antiplatelet regimen after stroke did not have a lower early recurrence rate than subjects who continued with the same antiplatelet therapy. In terms of safety outcomes, there was no difference in the occurrence of ICH. Conversely, patients in the continue group tended to have a lower though non-significant risk of ECH. Furthermore, the clinical outcomes were not significantly different across pre-defined subgroups. We found that change group was associated with an increase in the odds of a favourable functional outcome.

Currently, aspirin, a combination of aspirin/dipyridamole, clopidogrel and ticlopidine are recommended to prevent recurrent stroke in patients with stroke and TIA (Kernan, et al., 2014). In this study, we found that a single antiplatelet agent i.e. aspirin was frequently used in our cohort, followed by clopidogrel and a combination of aspirin/dipyridamole following ischaemic stroke. This may indicate the underutilization of combination antiplatelet therapy for prevention of recurrent stroke. The reason behind this probably because VISTA contains clinical trial data conducted during the years 1989 to 2006. Furthermore, the recommendation of clopidogrel alone and combination aspirin/dipyridamole are only become available after the European Stroke Prevention Study (ESPS-2) (Diener, et al., 1996) trial in 1996 and Prevention Regimen for Effectively Avoiding Second Strokes (ProFess) (Sacco, et al., 2008) trial in 2008.

There are no trials that show changed antiplatelet therapy after a stroke event reducing the risk of subsequent stroke (Kernan, et al., 2014). A recent retrospective cohort study by Lee, et al. (2014) compared clopidogrel initiation versus aspirin re-initiation among patients with ischaemic stroke who took aspirin at least 30 days prior to their index of stroke. They found those who changed to clopidogrel after a stroke was associated with fewer vascular events than aspirin. In contrast, we did not find any significant relation between those who change and continue antiplatelet therapy. The reasons might be because our study follow-up was shorter i.e. 90 days compared to study by Lee, et al.

(2014) and other related trials which are normally more than 2 years (Sacco, et al., 2008). Furthermore, we divided patients into two different groups, whether they change or continue with the same antiplatelet therapy and not according to specific antiplatelet agent. Although we repeated the analysis and compared between aspirin re-initiation vs clopidogrel initiation, the results are similar.

In this study, we tried to answer this question and the risks and benefits of either approach have not been well studied. In further analyses of this study, we found those who changed antiplatelet regimen or who changed from aspirin to clopidogrel was associated with good functional outcome at 90 days than those in continue group. There are limited trials compare clopidogrel and aspirin monotherapy and assessed functional outcomes such as mRS at 3 months in patients with stroke. In PRoFESS trial, the functional outcomes at 90 days were similar between clopidogrel and combination aspirin/dipyridamole treatment group (Sacco, et al., 2008). Whereas in ESPRIT trial, combination aspirin/dipyridamole has superior efficacy to aspirin monotherapy (Halkes, et al., 2006). Based on these two trials, we could indirectly assume that clopidogrel may be better than aspirin in prevention of recurrent stroke. Although the present study found better functional outcomes in change group, this might be due to confounding with other variables at the baseline. There are differences exist between change and continue groups. In change group, stroke severity and patients with atrial fibrillation at baseline were significantly lower than continue group and these two factors are known to associate with an increased risk of poor outcome following an ischaemic stroke (Muir, et al., 1996; Adams, et al., 1999; Andersen, et al., 2011; Saposnik, et al., 2011; McGrath, et al., 2013).

There are several limitations to this study. The analysis was performed using a non-randomized registry data that derived from various clinical trials. Thus, selection and other biases could have confounded antiplatelet therapy and the decision to stop and continue. The number of included participants was low due to the selection criteria and need to be on antiplatelet therapy before and after a stroke. Although the recurrent stroke rate was identical and lower between groups, we lack statistical power to exclude a potentially clinically significant difference between groups. Revised antiplatelet therapy was associated with a

lower rate of bleeding that did not reach statistical significance. There are studies suggesting differences in stroke recurrence while on antiplatelet therapy based on stroke subtype. However, we were unable to investigate this association due to the information is not available in most of the patients. On the other hand, study subjects were not randomized and the duration and the dose of antiplatelet, and the extent of medication adherence for antiplatelet agent before and after a stroke were not available. The interpretation of the study data might be more meaningful if we have more information on certain aspects of medical care such as use of lipid-lowering agents, diabetic and hypertensive control or lifestyle changes.

In this study, the analyses were adjusted for age and baseline severity. These variables are the two most powerful factors influence stroke prognosis and are commonly included in analyses of the distribution of functional outcomes (Lees, et al., 2006; Konig, et al., 2008; Bath, et al., 2012). We also provided additional analyses adjusted for every patient characteristic that differed between groups at baseline. Subgroup analyses in patients with and without prior stroke and atrial fibrillation were also included.

There are also strengths in the present study. The data are derived from prospective trials with standardized assessments and careful data monitoring, providing high quality data. In addition, we were able to adjust for the most important prognostic variables.

3.5 Conclusion

In summary, in patients who suffer ischaemic stroke whilst taking antiplatelet, a change of antiplatelet regimen was not associated with a lower risk of recurrence than continuing on the same antiplatelet regimen. Despite no clear evidence for changing antiplatelet strategies following a cardiovascular event, over 50% of patients had their medication changed. Although, the results might be applicable to clinical practice, as recommended by the guideline, the selection of antiplatelet regimen after ischaemic stroke should be individualized on the basis of patient risk factor profiles, cost, tolerance, relative known efficacy of the agents and other clinical characteristics (Kernan,

et al., 2014). However, this was a small retrospective analysis and future prospective trials will be needed.

4 Association of between outcomes and the timing of antiplatelet therapy after ischaemic stroke

4.1 Introduction

Patients with ischaemic stroke have high risk of recurrent stroke, which often occurs in the first few days after the index stroke (Dhamoon, et al., 2006; Giles and Rothwell, 2007; Rothwell, et al., 2007; Mohan, et al., 2011). Guidelines suggest initiation of antiplatelet therapy within 24 to 48 hours after ischaemic stroke onset, unless thrombolytic therapy has been administered where it must be avoided for 24 hours. In the US, the American Stroke Association guidelines suggest initiation of aspirin within 24 to 48 hours after stroke onset (Jauch, et al., 2013). The National Institute for Health and Care Excellence (NICE) guideline recommends initiation of the anti-platelet aspirin within 24 hours (National Institute for Health and Clinical Excellence, 2008) and the European Stroke Organisation (ESO) guidelines suggest aspirin be given within 48 hours after ischaemic stroke (Hacke, et al., 2008b). The recommendations are based on data from two large trials, the Chinese Acute Stroke Trial (CAST) and International Stroke Trial (IST), where outcomes were improved with aspirin therapy, commenced within the first 48 hours (Chen, et al., 1997; International Stroke Trial Collaborative Group, 1997).

Dual antiplatelet therapy is recommended to be given immediately following the diagnosis of ACS (National Institute for Health and Care Excellence, 2013). Early initiation of antiplatelet therapy in ACS safely reduces mortality, recurrent myocardial infarction and major vascular events (Yusuf, et al., 2001; Chen, et al., 2005; Sabatine, et al., 2005b). In CURE trial, although major bleeding was significantly higher in DAPT treatment group, there was no excess in bleeding that leads to haemorrhagic stroke compare to aspirin monotherapy group (Yusuf, et al., 2001). Based on these trials, we could indirectly infer that there are benefits of early initiation of antiplatelet therapy immediately after ischaemic stroke.

The initiation of clopidogrel and aspirin within 24 hours and continued for 3 weeks reduces recurrent stroke with acceptable risk in comparison to aspirin mono-therapy in patients with high-risk transient ischaemic attack or minor ischaemic stroke (Wang, et al., 2013). This suggests there are benefits to very early initiation (<24 hours after ischaemic stroke) of antiplatelet drugs, but whether these benefits extend to very early initiation of antiplatelet drugs in the wider stroke population is not known.

The present study assessed recurrent ischaemic stroke rate and bleeding complications associated with early or later initiation of anti-platelets using data from the Virtual International Stroke Trials Archive (VISTA).

4.2 Methods

4.2.1 Study setting and study population

General details of the study setting and study population are described in section 2.2.1. Briefly, the data used are contained in the VISTA database. The VISTA data are anonymous to patients and clinical trials.

4.1.1 Definition of antiplatelet Exposure

In VISTA, data on antiplatelet treatment were based on platelet aggregation inhibitors with ATC code: B01AC (which includes derivatives of salicylic acid, thienopyridine and dipyridamole). Details on data extraction, cleaning and coding as described in Chapter 2 (section 2.2.1).

Patients were divided into two different groups based on when they commenced their antiplatelet therapy. Medication data on antiplatelet therapy in VISTA were recorded as the date the medication was started or first given. Patients were defined as early when they initiated antiplatelet therapy on the same or following day after stroke; day 0 or 1 and late; day 2 or later. The late group was further divided into mid-time initiation (defined as antiplatelet

initiation on day 2 or 3 post stroke) and late initiation (antiplatelet initiation on day 4 or later). Detailed of study flow chart is presented in Figure 4.1.

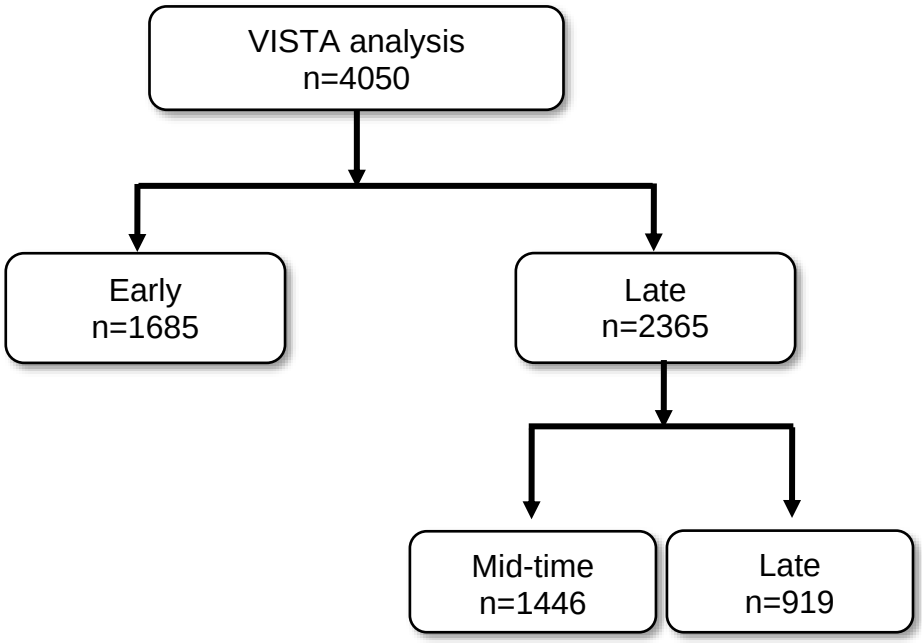


Figure 4-1: Flow diagram describing the selection of data from the Virtual International Stroke Trials Archive for the analysis reported.

4.2.2 Outcome assessment

The primary efficacy outcome was recurrent ischaemic stroke within 90 days. The primary safety outcome was intracranial haemorrhage (ICH) and the secondary safety outcome was extracranial haemorrhage (ECH). The modified Rankin Scale (mRS) at 90 days was used to measure the functional outcomes. Definition and data extraction for each outcome measures as described in Chapter 2 (section 2.2.1 and section 2.3.1).

4.2.3 Statistical analysis

Descriptive statistics were recorded for recent stroke patients, comparing two groups (early vs late) and three groups (early vs mid-time vs late) of antiplatelet initiation. For categorical variables, such as demographics and medical history data, they were summarised using frequencies and proportions and were compared using the Chi-square test or Fisher's exact test, where appropriate. Whereas for continuous variables, they were summarised using mean [standard deviation (SD)] or median [interquartile range (IQR)] and were compared using Student t-test or non-parametric Mann-Whitney test when comparing two groups and one-way analysis of variance (ANOVA) or non-parametric Kruskal Wallis test when comparing three groups.

The occurrence of recurrent stroke, ICH and ECH within 90 days were compared between the early and late group; and between the early, mid and late time antiplatelet initiation. The odds ratio (OR) of achieving the studied outcome(s) against the comparator, and corresponding 95% confidence intervals (95% CI), were calculated using logistic regression. Early initiation group was considered as the reference group. To account for potential immortal time bias in exposure duration (Lévesque, et al., 2010; Liu, et al., 2012), bleeding complications analysis was repeated excluding bleeding events within the first four days post stroke. We then explored the effect of prior antiplatelet therapy and thrombolytic therapy. We also measured the ordinal shift of the functional outcome i.e. mRS at day 90 using the full scale and we calculated the OR (95% CI) using ordinal logistic regression for mRS distribution.

The effect of between three groups on the outcome measures in subgroup: prior antiplatelet therapy and thrombolytic therapy were assessed. Subgroup analyses in these populations were conducted as their stroke risk outcome (Bejot, et al., 2013) and neurological recovery (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995) are different.

All models were adjusted for age and baseline National Institutes of Health Stroke Scale (NIHSS) (Konig, et al., 2008; Bath, et al., 2012). A $p<0.05$ was

considered significant. All analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp, 2012), whereas forest plots were produced using StatsDirect Statistical Software version 3.0.171.

4.3 Results

4.3.1 Patients sample

There were 4050 acute ischaemic stroke patients included. Of these 1685 (41.6%) patients were in the early group and 2365 patients in the late group. The late initiation group which was further divided consists of 1446 (35.7%) in the mid-time group and 919 (22.7%) late patients (Figure 4-1).

Patients who were received late antiplatelet therapy had more severe stroke symptoms at the baseline and had a lower prevalence of diabetes, ischaemic heart disease and prior stroke compared with the early group (Table 4-1). Patients who received mid-time antiplatelet therapy had more severe stroke symptoms and had a higher prevalence of heart failure compared with the early group (Table 4-2). Patients in the late group were more likely to have been previously treated with antiplatelet and have atrial fibrillation and ischaemic heart disease than those in the early group. More than 90% of patients who received thrombolytic therapy were in mid-time and late groups.

Table 4-1: Baseline characteristics comparing 2 groups

Characteristics	Initiation of AP		p-value
	Early n=1685	Late n=2365	
Age, years*	68.32 (12.521)	67.73 (12.756)	0.215
Male	946 (56.1)	1355 (57.3)	0.466
Baseline NIHSS†	11 (8-16)	13 (9-17)	0.000
Prior AP	460 (27.3)	669 (28.3)	0.490
Thrombolysed, rt-PA	137 (8.1)	1220 (51.6)	0.000
Medical History			
Hypertension‡	1171/1660 (70.5)	1605/2295 (69.9)	0.680
Diabetes‡	405/1684 (24.0)	455/2365 (19.2)	0.000
Atrial fibrillation‡	232/1660 (14.0)	326/2295 (14.2)	0.838
Heart failure‡	88/1519 (5.8)	138/2045 (6.7)	0.247
Ischaemic heart disease‡	399/1577 (25.3)	500/2232 (22.4)	0.038
Previous TIA‡	137/1583 (8.7)	174/2195 (7.9)	0.422
Previous stroke‡	304/1581 (19.2)	346/2101 (16.5)	0.030

All values are reported as no. (%) unless otherwise noted. *Values are reported as mean (SD); †median (IQR); ‡missing some data at baseline. AP=Antiplatelet, IQR=interquartile range, NIHSS=National Institutes of Health Stroke Scale, rt-PA=recombinant tissue plasminogen activator, SD=standard deviation, TIA=transient ischaemic attack.

Table 4-2: Baseline characteristics comparing 3 groups

Characteristics	Initiation of AP			p-value
	Early n=1685	Mid-time n=1446	Late n=919	
Age, years*	68.32(12.52)	67.51(13.05)	68.07(12.29)	0.319
Male	946 (56.1)	823 (56.9)	532 (57.9)	0.688
Baseline NIHSS†	11(8-16)	13(9-18)	12(8-17)	0.000
Prior AP	460 (27.3)	382 (26.4)	287 (31.2)	0.031
Thrombolysed, rt-PA	137 (8.1)	885 (61.2)	335 (36.5)	0.000
Medical History				
Hypertension‡	1171/1660 (70.5)	983/1411 (69.7)	622/884 (70.4)	0.863
Diabetes‡	405/1684 (24.0)	277/1446 (19.2)	178/919 (19.4)	0.001
Atrial fibrillation‡	232/1660 (14.0)	180/1411 (12.8)	146/884 (16.5)	0.041
Heart failure‡	88/1519 (5.8)	86/1297 (6.6)	52/748 (7.0)	0.492
Ischaemic heart disease‡	399/1577 (25.3)	269/1382 (19.5)	231/850 (27.2)	0.000
Previous TIA‡	137/1583 (8.7)	113/1350 (8.4)	61/845 (7.2)	0.459
Previous stroke‡	304/1581 (19.2)	206/1333 (15.5)	140/768 (18.2)	0.026

All values are reported as no. (%) unless otherwise noted. *Values are reported as mean (SD); †median (IQR); ‡missing some data at baseline. AP=Antiplatelet, IQR=interquartile range, NIHSS=National Institutes of Health Stroke Scale, rt-PA=recombinant tissue plasminogen activator, SD=standard deviation, TIA=transient ischaemic attack.

4.3.2 Clinical outcomes

Table 4-3 shows the incidence of the primary outcome and safety outcomes in early and late groups. The incidence of recurrent stroke was no different between early and late initiation group (adjusted OR for late group 1.12; 95% CI, 0.80-1.59; p=0.510). On the other hand, compared to early group, the odds of ICH were lowered by 35% (adjusted OR, 0.65; 95% CI, 0.42-0.995; p=0.047 for ICH) and the odds of ECH were lowered by 42% (adjusted OR, 0.58; 95% CI, 0.41-0.83; p=0.003) in the late group. There was no significant difference when only bleeding events after day 4 were considered (Table 4-4).

Figure 4-2 shows the incidence of the primary outcome and safety outcomes in early, mid-time and late initiation groups. The incidence of recurrent stroke was no different (adjusted OR compared to early group, 1.00; 95% CI, 0.67-1.49; p=0.988 for mid-time and 1.31; 95% CI, 0.86-1.99; p=0.208 for late group respectively). There was no excess risk of bleeding in the mid-time group compared to the early group but the late group had the lowest rate of ICH (adjusted OR for late group 0.35; 95% CI, 0.17-0.72; p=0.005 for ICH and adjusted OR, 0.29; 95% CI, 0.16-0.55; p=0.000 for ECH respectively). There was no significant difference when only bleeding events after day 4 were considered (Figure 4-3).

Table 4-3: Clinical outcomes at Day 90 (adjusted for age and baseline NIHSS)

Outcomes	Early n=1685	Late n=2365	Adjusted OR (95% CI)	p-value
Recurrent stroke	56/1685 (3.3)	74/2353 (3.1)	0.96 (0.67-1.37)	0.807
ICH	43/1685 (2.6)	43/2323 (1.9)	0.65 (0.42-0.995)	0.047
ECH	71/1684 (4.2)	62/2316 (2.7)	0.58 (0.41-0.83)	0.003

OR is the odds of achieving a 1 on the specified outcome. Early as reference group. CI=confidence interval, ECH=extracranial haemorrhage, ICH=intracranial haemorrhage, OR=odds ratio, n=number of observations.

Table 4-4: Bleeding complications at Day 90 (excluded event before ≤ day 4) between two groups

Outcomes	n (%)	Adjusted OR (95% CI)	p-value
Intracranial haemorrhage			
Early n=1685	14/1656 (0.8)	-	0.556
Late n=2365	26/2306 (1.1)	1.22 (0.63-2.35)	
Extracranial haemorrhage			
Early n=1685	23/1636 (1.4)	-	0.842
Late n=2365	37/2291 (1.5)	1.06 (0.62-1.79)	

All models were adjusted for age and baseline NIHSS. OR is the odds of achieving a 1 on the specified outcome. Early as reference group. CI=confidence interval, OR=odds ratio.

Outcomes	n (%)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	p-value
Recurrent stroke				
Early n=1685	56/1685 (3.3)		1.00 (0,0)	
Mid-time n=1446	45/1444 (3.1)		0.95 (0.64-1.42)	0.802
Late n=919	29/909 (3.2)		0.97 (0.61-1.53)	0.886
ICH				
Early n=1685	43/1685 (2.6)		1.00 (0,0)	
Mid-time n=1446	34/1437 (2.4)		0.83 (0.52-1.31)	0.422
Late n=919	9/886 (1.0)		0.35 (0.17-0.72)	0.005
ECH				
Early n=1685	71/1684 (4.2)		1.00(0,0)	
Mid-time n=1446	50/1435 (3.5)		0.76 (0.52-1.10)	0.150
Late n=919	12/881 (1.4)		0.29 (0.16-0.55)	0.000

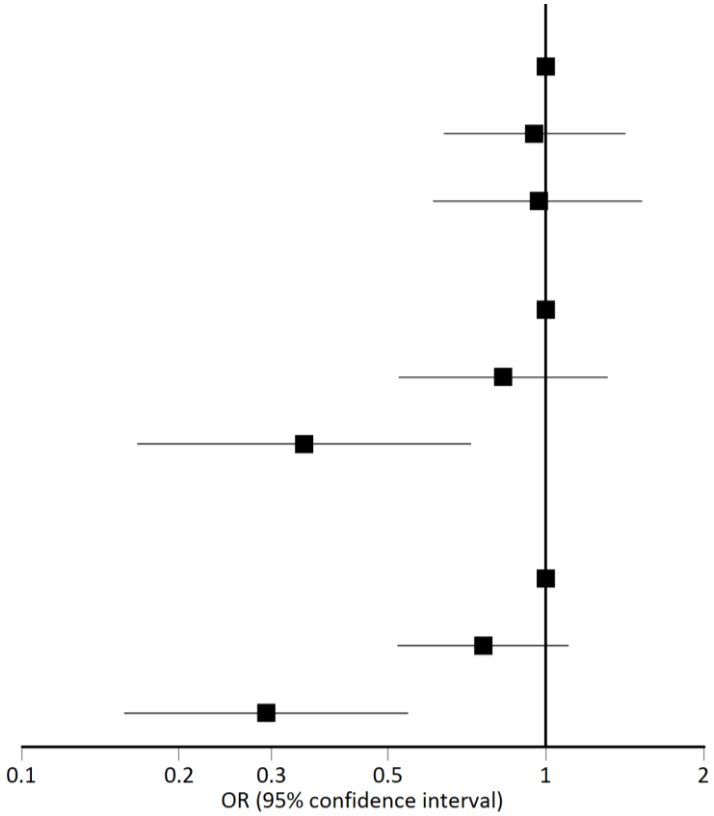


Figure 4-2: Clinical outcomes at Day 90 comparing 3 groups (adjusted for age and baseline NIHSS).

OR is the odds of achieving a 1 on the specified outcome. Early as reference group. CI=confidence interval, ECH=extracranial haemorrhage, ICH=intracranial haemorrhage, OR=odds ratio.

Outcomes	n (%)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	p-value
Intracranial haemorrhage				
Early n=1685	14/1656 (0.8)		1.00 (0,0)	
Mid-time n=1446	18/1421 (1.3)		1.37 (0.68 - 2.77)	0.385
Late n=919	8/885 (0.9)		0.97 (0.40 - 2.35)	0.954
Extracranial haemorrhage				
Early n=1685	23/1636 (1.4)		1.00 (0,0)	
Mid-time n=1446	26/1411 (1.8)		1.20 (0.68 - 2.21)	0.530
Late n=919	11/880 (1.3)		0.82 (0.39 - 1.70)	0.590

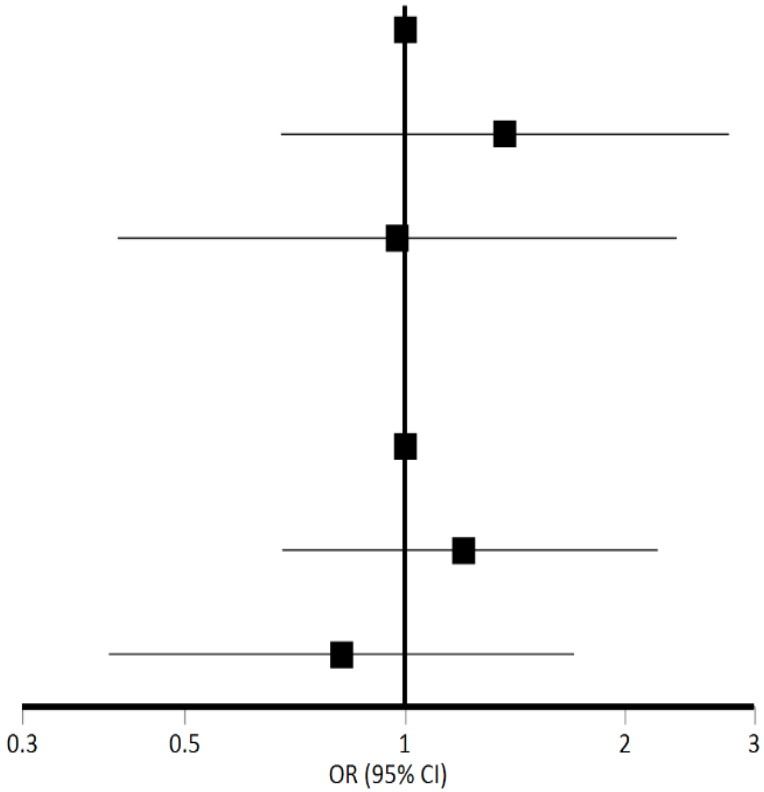


Figure 4-3: Bleeding complications at Day 90 (excluded event before ≤ day 4) between three groups.

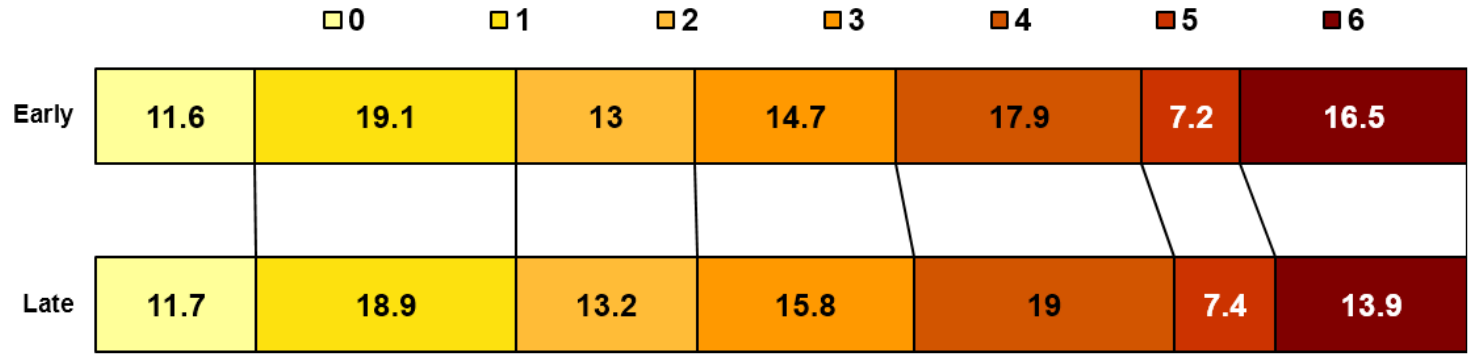
All models were adjusted for age and baseline NIHSS. OR is the odds of achieving a 1 on the specified outcome. Early as reference group. OR=odds ratio, CI=confidence interval, n=number of observations.

4.3.3 Functional outcomes

The distribution of mRS at 90 days comparing two and three groups are presented in Figure 4-4. We found that the late initiation was associated with a significantly more favourable functional outcome across full scale mRS at 90 days after adjustments for age and baseline NIHSS (OR, 1.31; 95% CI, 1.17-1.47), compared with the early initiation group Figure 4-4(a).

When comparing three groups, mid-time initiation was associated with more favourable distribution of mRS scores at 90 days (adjusted OR 1.32; 95% CI 1.16-1.50), compared with the early initiation group. Late initiation was associated with a significantly better functional outcome across full scale mRS at 90 days (adjusted OR 1.15; 95% CI 1.07-1.24), compared with the early initiation group Figure 4-4(b).

(a) Early vs late initiation group



(b) Early vs mid-time vs late initiation group

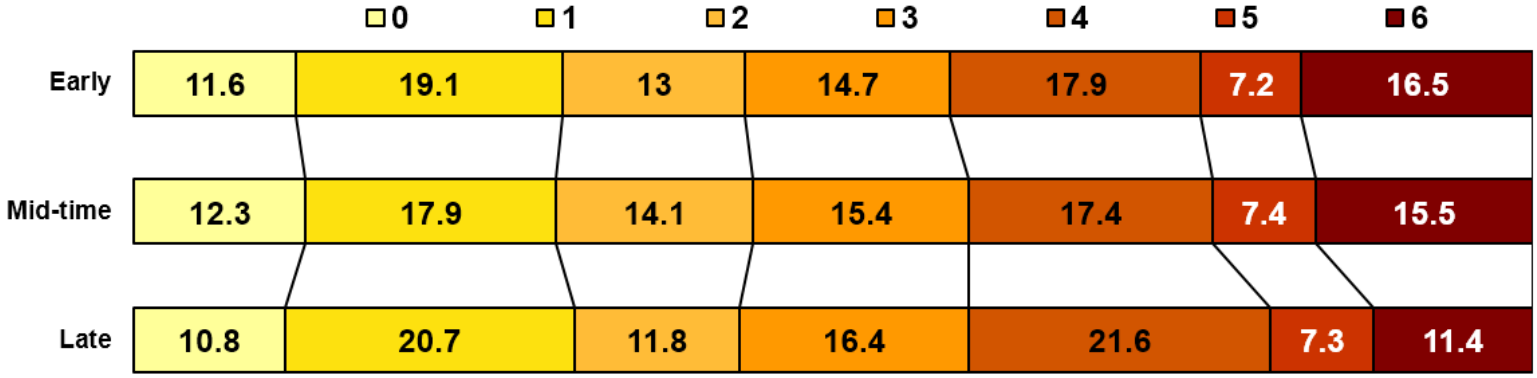
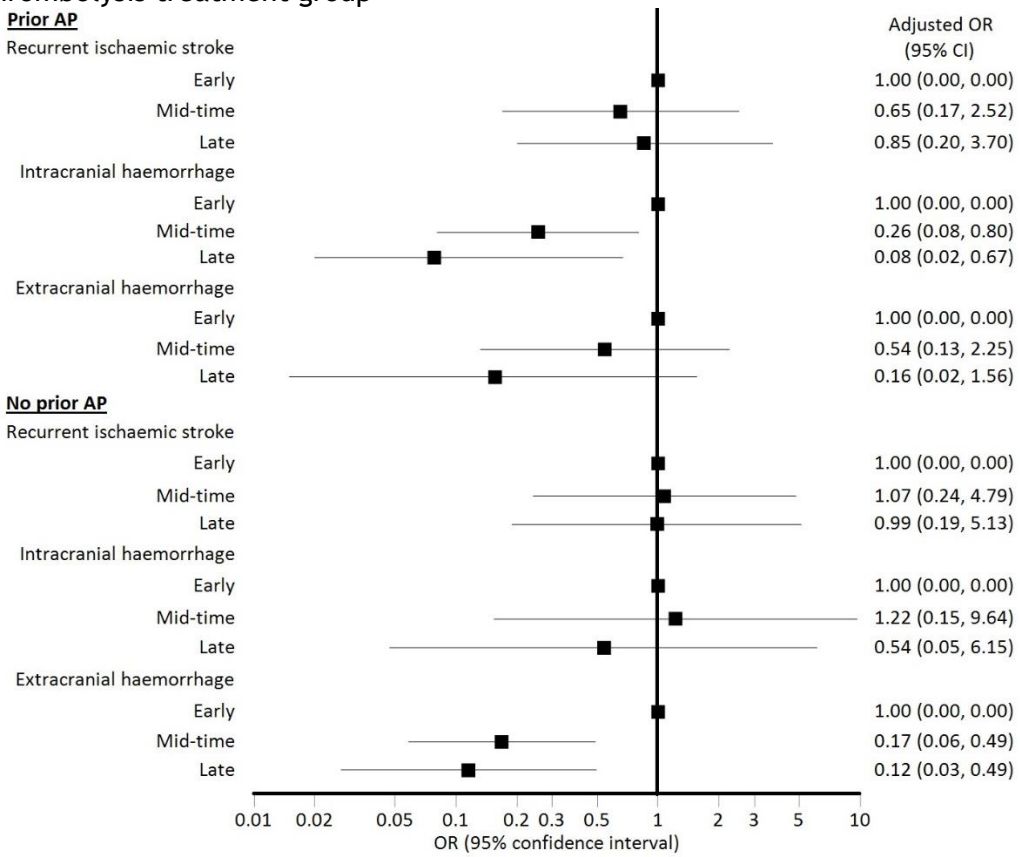


Figure 4-4: Distribution of mRS outcome at day 90 by antiplatelet initiation group (a) compared two groups, (b) compared 3 groups.
All models were adjusted for age and baseline NIHSS.

4.3.4 Subgroup analysis

Subgroup analyses by previous antiplatelet therapy and thrombolytic therapy are shown in Figure 4-5. However, we did not find a significant influence on the occurrence of recurrent stroke, ICH and ECH event between early, mid-time and late groups in these populations. The results were broadly similar.

(a) Thrombolysis treatment group



(b) Non-thrombolysis treatment group

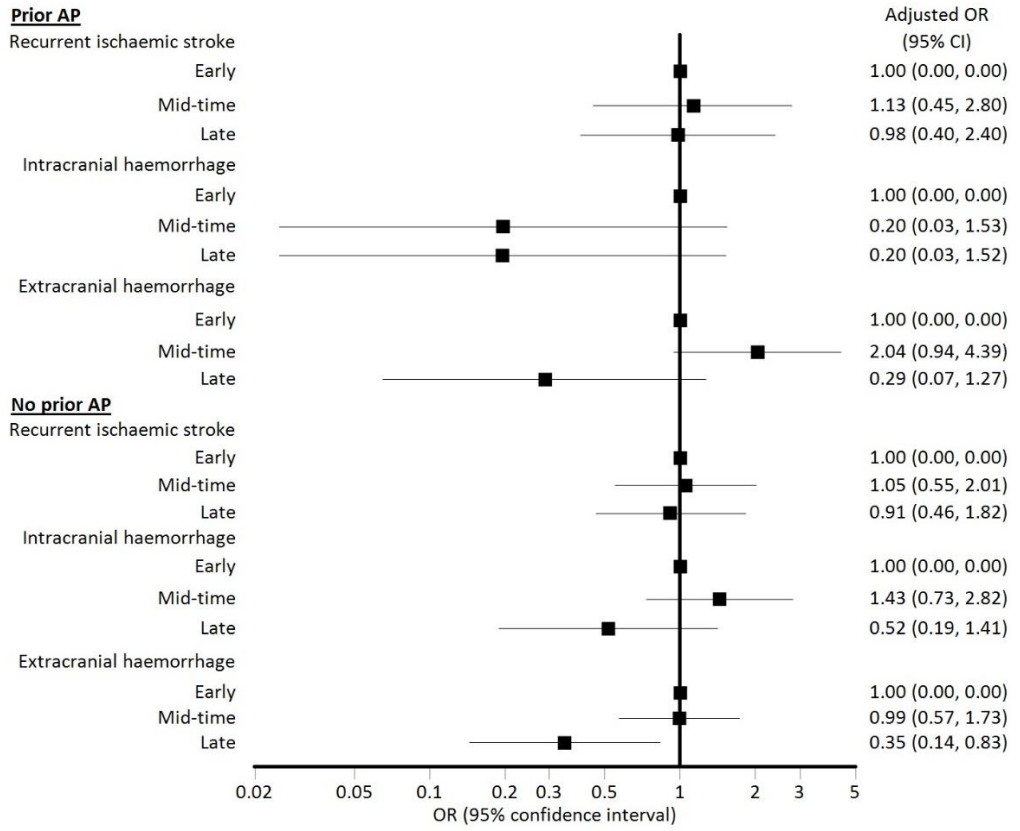


Figure 4-5: Forest plot showing outcomes for (a) thrombolysis and (b) non-thrombolysis treatment group with and without prior antiplatelet therapy.

Early as a reference group. All models adjusted to age and baseline NIHSS. OR is the odds of achieving a 1 on the specified outcome. Early as reference group. AP=antiplatelet, CI=confidence interval, OR=odds ratio.

4.4 Discussion

We explored the relationship between time of antiplatelet initiation following acute ischaemic stroke and risk of recurrent stroke and bleeding complications. There was no difference in recurrent stroke risk, but later introduction of antiplatelet therapy (on or after day 4) was associated with the lowest risk of bleeding complications. However, the finding should be interpreted with caution as they are based on retrospective analysis of data and likely reflect exposure bias.

The incidence of recurrence ischaemic stroke in the present study was 3.3% in the early group which is slightly higher to that seen in previous trials (2.8%) (1997). Similarly, it is higher than the rate in the CAST trial (1.6% vs 2.1% in aspirin and placebo treated patients) (Chen, et al., 1997). This may reflect differences in the trial populations and also the duration of follow up (90 days in our study vs 4-weeks in CAST). The rate of recurrent events was lower in the present study than in the recent CHANCE trial (where the rate was 8.2% and 11.7% in the aspirin plus clopidogrel and aspirin only groups) (Wang, et al., 2013). The CHANCE trial included patients with minor stroke and high risk transient ischaemic attack who are presumably at higher risk than our study sample.

In this study, the rates of recurrent stroke were particularly low in the early initiation of antiplatelet therapy. Subsequently, the rates of recurrent stroke increased when initiation of antiplatelet therapy within 48 to 72 hours and more than 72 hours. Although the rates of recurrent stroke were low in the early initiation, there was no statistically significant different compared to later initiation. In clinical practice, it is important to start antiplatelet therapy as soon as possible after the onset of stroke symptom because the rate of ischaemic event is higher within 24 hours after symptoms appear. Therefore, further analyses are required to understand the true clinical impact of early initiation of antiplatelet therapy.

In this study, we found that initiation of antiplatelet therapy within the first three days after acute ischaemic stroke, was associated with an increased incidence of bleeding complications. We found the ICH incidence rate was 2.1% which was

higher compared with other trials (Chen, et al., 1997; Wang, et al., 2013). Patients age is a known predictor of ICH (Ariesen, et al., 2006; Berger, et al., 2010; Graipe, et al., 2015) and the present study included older patients (over 65 years old) than previous studies (mean age between 62 and 63 years old). Furthermore, our study included patients with more severe stroke (baseline NIHSS 13) and at higher risk than previous study (baseline NIHSS ≤ 3). As the immortal time bias could inflate the incidence rates for ICH and ECH, we circumvent this issue by repeated the bleeding complication analyses and omitting bleeding events occurring after day 4 and we found the trend toward increased bleeding was attenuated.

This study aimed to assess the impact of earlier initiation of antiplatelet therapy in order to provide data to inform guideline recommendations which are limited by available data. Early initiation of antiplatelet therapy for the treatment of acute ischaemic stroke has been well established. In CAST trial, a large randomized controlled trial, have shown that initiation of aspirin within 48 hours in patients with suspected acute ischaemic stroke reduces the immediate risk of further stroke or death in hospital and the overall risk of death or dependency (Chen, et al., 1997). Additionally, in IST trial, patients treated with aspirin within 48 hours stroke symptoms had a lower recurrence rate within 14 days with no increased risk of haemorrhagic strokes (1997). A systematic review (N=43,041) of antiplatelet therapy for acute stroke concluded that initiation of aspirin within 48 hours has been shown to reduce the risk of recurrent ischaemic stroke without major risk of early bleeding complications, but also improved long-term outcome (Sandercock, et al., 2008).

The results of this study, i.e. the occurrence of recurrent stroke, ICH, ECH and functional outcomes differ from those of other trials of antiplatelet therapy with aspirin and clopidogrel or dipyridamole after cerebral ischaemic events (Kennedy, et al., 2007; Dengler, et al., 2010; Wang, et al., 2013). In this observational study, we assessed early use of antiplatelet therapy within 24 hours, 72 hours and beyond 72 hours after stroke. In the analysis, we included population with all types of stroke severity and those who had received all types of antiplatelet that can be combination or single antiplatelet therapy. Previous studies included patients with less severe strokes and they either compare dual antiplatelet to single or single antiplatelet to placebo in patients within 24 hours after an acute minor stroke or

TIA. This might be the reason in this study did not show a reduction in the risk of ischaemic events, but at the same time showed an increased risk of haemorrhage.

We also found that in patients treated with thrombolytic therapy that early use of antiplatelet was associated with higher bleeding risk, particularly in patients who were taking antiplatelet before their stroke. This supports the current practice of delaying antiplatelet therapy for at least 24 hours after thrombolysis in ischaemic stroke (Zinkstok and Roos, 2012). In patients with TIA and minor stroke, early and aggressive antiplatelet treatment appears to be beneficial (Bath, et al., 2015). In the present study, the median baseline NIHSS scores in study groups ranged from 11 to 13. Whether very early and / or enhanced antiplatelet therapy is of benefit in ischaemic stroke more widely requires investigation in well-designed randomised trials. Our data support the assertion that the risk benefit ratio is fine and extrapolation cannot be made from studies of milder phenotypes.

Analyses in this chapter also demonstrated that mid-time and late initiation of antiplatelet therapy after acute stroke are associated with better functional outcomes. However, this observational study can be only seen as suggestive and must be interpreted with caution. There were differences at baseline, the comorbidity which known to have association with unfavourable functional outcome, i.e. atrial fibrillation and/or diabetes were all less prevalent in mid-time and late initiation groups. Furthermore, thrombolysis is generally associated with good outcome after ischaemic stroke and in this study, this treatment was common in mid-time and late initiation groups than early group.

There are also limitations to our study that must be considered. The analysis of this study was performed using a non-randomized registry data that derived from various clinical trials. Thus, it is inevitably incorporates confounders. Baseline characteristics can differ between groups due to selection bias. In contrast, we have adjusted our analyses for the most important factors i.e age and baseline stroke severity (Lees, et al., 2006; Konig, et al., 2008). Due to lack of data, we were unable to adjust for every factor that associated with stroke risk recurrence such as dysphagia and stroke subtype. In the early stage, we planned to adjust for all variables that differed at baseline. However, on trying to adjust with the

variables that differed at baseline, in multivariable analysis model for recurrent stroke, only one variable reach significant difference for example, diabetes and all other important variables such as age and baseline NIHSS were excluded from the model. Furthermore, the size sample for the analysis was reduced because observations were removed by the statistical program due to missing values for the related variables. Alternatively, we provided subgroup analyses in patients with and without prior antiplatelet and thrombolytic therapy.

Even though VISTA trials used standardized approaches for data collation, we were unable to compensate fully for all the differences. Random error within variables is likely as the definition might be varied in different trials. As data in VISTA were anonymized for trial source, we were unable to access specific definitions for each variable from the pooled databases. The outcome events used in this study (recurrent stroke, ICH and ECH), were based on AE and SAE reports as described in the Methods section, rather than performing the neurologic examination and brain imaging to confirm the diagnosis of ischaemic stroke. Therefore, it is possible that our estimates of events are less precise in terms of time. The non-random allocation of antiplatelet therapy is a flaw. We were unable to determine the rationale for each treatment decisions and delays for patients from our database. The clinical decision on when to initiate antiplatelet therapy depends on the individual clinician's perception of benefits and risk.

4.5 Summary

In summary, early initiation of antiplatelet therapy was not associated with better outcomes than later initiation in patients who have suffered ischaemic stroke, although our analysis is underpowered. Furthermore, there was an increased risk of bleeding with early initiation.

5 Interruption to antiplatelet therapy early after acute ischaemic stroke: Nested case-control study

5.1 Introduction

Antiplatelet therapy is recommended after acute ischaemic stroke to reduce the risk of recurrent ischaemic stroke and other vascular outcomes (Gent, et al., 1996). Guidelines suggest use of either aspirin, clopidogrel or the combination of aspirin/dipyridamole (National Institute for Health and Clinical Excellence, 2008; National Institute for Health and Clinical Excellence, 2010; Furie, et al., 2011). However, persistence with antiplatelet regimens is variable after stroke. The reported rates of aspirin discontinuation vary from less than 10% to almost 50% (Sud, et al., 2005; Lago, et al., 2006), and data on adherence early after stroke are limited.

Evidence shows that interrupting or stopping antiplatelet therapy increases the risk of cardiovascular events (Ferrari, et al., 2005; García Rodríguez, et al., 2011). However, cessation of antiplatelet drugs may of course be justified and required due to withdrawal of care, bleeding episodes or other adverse drug reactions. This is particularly true in the period early after ischaemic stroke where recurrence rate is also highest. Data to demonstrate the impact of stopping anti-platelets early after ischaemic stroke are lacking. We aimed to assess whether, in patients who have suffered ischaemic stroke, early cessation, stopped or interrupted of antiplatelet is associated with an increased risk of cardiovascular event than persistent antiplatelet regimen.

5.2 Methods

5.2.1 Study setting and study population

This study used data of acute ischaemic stroke patients within the study cohort derived from the Virtual International Stroke Trials Archive (VISTA) database.

General details of the study setting and study population are described in section 2.2.1. The study cohort included information on patient characteristics, medical history, medication history and the occurrence of adverse events (AE) and serious adverse events (SAE).

5.2.2 Study design

A matched case-control study design was used to examine association between antiplatelet exposure and the cardiovascular event.

5.2.3 Outcome

The outcome of the study was cardiovascular event within 90 days following acute ischaemic stroke. This includes recurrent ischaemic stroke, ACS and TIA. The event was identified from AE and SAE reports datasets which has been described in section 2.2.1.

5.2.4 Case definition

Cases were defined as patients who had at least one cardiovascular event in the first 90 days after acute ischaemic stroke. Patients who had a cardiovascular event within the first two days after ischaemic stroke were excluded as the event might not be associated to antiplatelet but due to the specific pattern of ischaemic changes after acute stroke (Wasserman, et al., 2014). Figure 5-1 shows the flowchart of patient's selection.

5.2.5 Control definition

Controls were defined as patients with acute ischaemic stroke, but had no cardiovascular event within 90 days after acute ischaemic stroke. The aim was to find a matched control to make the two groups as similar as possible. Selecting more than one control per case can increase the statistical power of a matched case-control study (Ahrens, et al., 2014). However, there is no additional gain in power and it was not worth matching to more than 4 controls per case (Pang,

1999; Ahrens, et al., 2014). For these reasons, the decision was taken to select up to four controls per case.

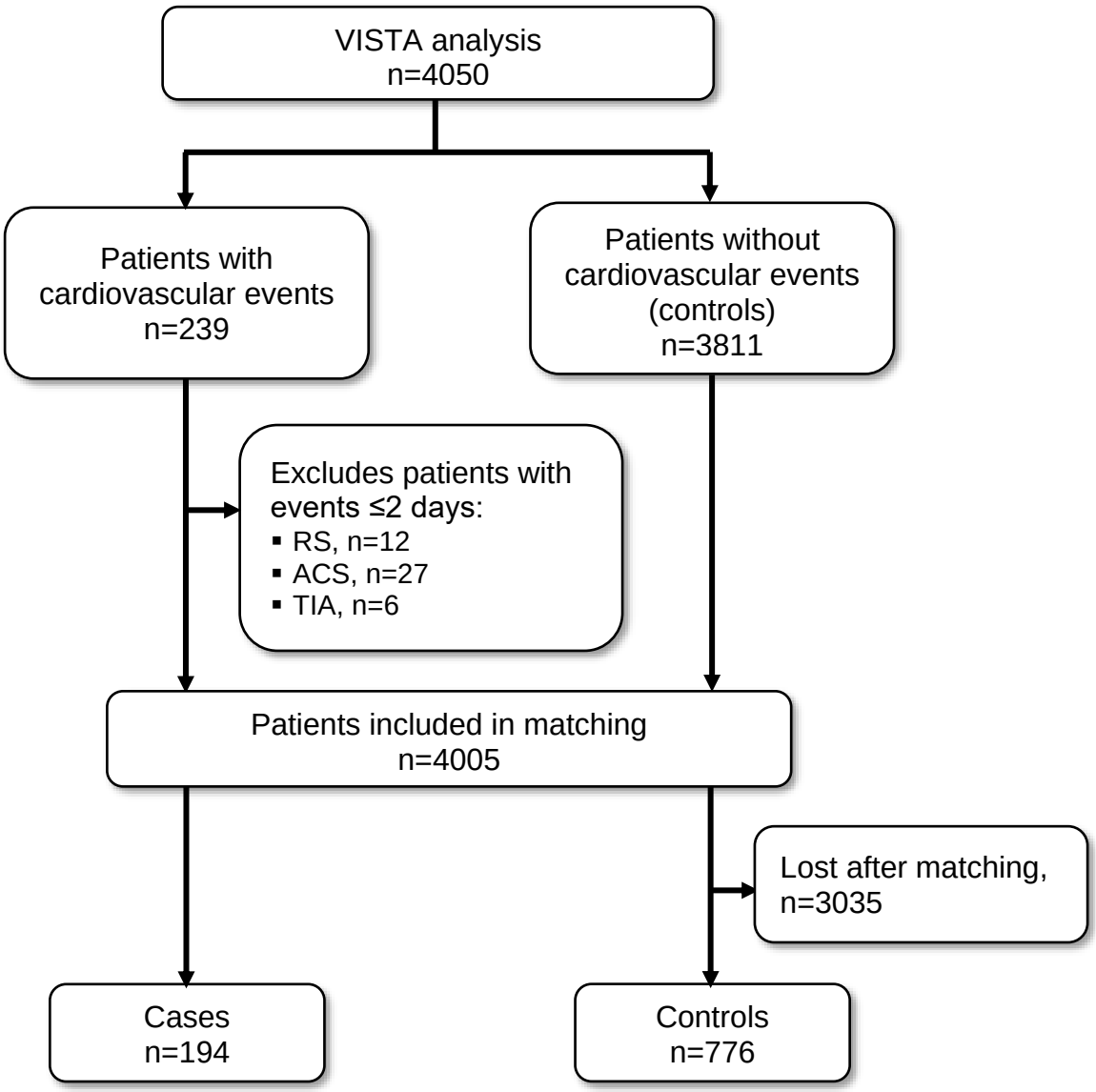


Figure 5-1: Flowchart of patients' selection.

ACS=acute coronary syndrome; RS=recurrence stroke; TIA=transient ischaemic attack.

5.2.6 Case-control matching

Matching in a case-control study refers to the procedure of selection of controls for each case on certain characteristics in order to make the two groups as comparable as possible (Breslow, 2014).

An algorithm, the automating selection of controls matching programme from Mounib and Satchi (2000), was adapted to carry out one-to-four individual matching of cases to controls. Two matching criteria were chosen because each was found to be associated with the risk of recurrent stroke (Pennlert, et al., 2014) and were thus potential confounders: i) age (± 10 years) and ii) sex. The multiple variables matching programme used is shown in **Appendix 8**. At the end of the matching process, four controls per case were selected at random without replacement. The process generated matched sets, each comprising a single case and four controls.

5.2.7 Exposures

The exposure period for each patient began after the diagnosis of acute ischaemic stroke and ended at the index date. The index date was defined as follows: for cases, it was the first cardiovascular event recorded after the antiplatelet exposure; for controls it was the same date as the matched case (Hubbard, et al., 2002; Garbe and Suissa, 2014). Figure 5-2 shows the exposure period for a hypothetical case and matched control.

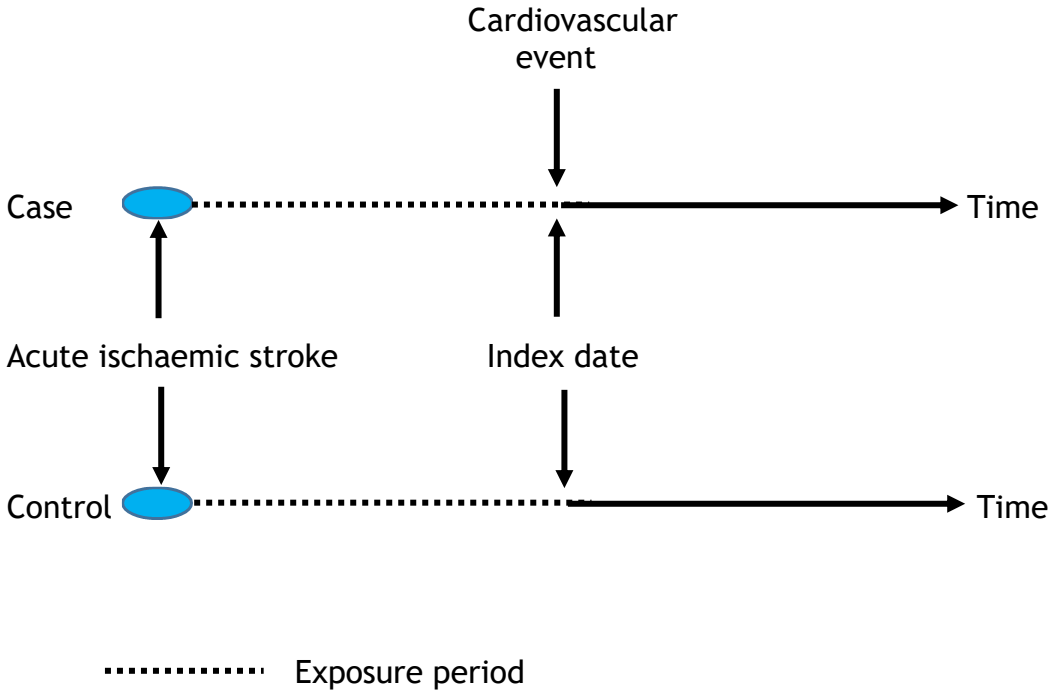


Figure 5-2: The exposure period for hypothetical case and matched control: from the diagnosis of acute ischaemic stroke to the index date.

5.2.8 Antiplatelet drug exposure

Cases and controls were further categorised based on exposure to antiplatelet drugs after acute ischaemic stroke and up to the index date. Each individual was classified as a persistent user, early cessation user, interrupted user and stopped user (Figure 5-3). Persistent users were patients who still took antiplatelet therapy three days and more before the index date. Early cessation user was defined as patient who took antiplatelet therapy less than three days prior to the index date. Patients with two days interruption of antiplatelet but still took at least two days before the index date were classified as interrupted users. Patients who stopped antiplatelet at least 5 days before the index date were identified as stopped users.

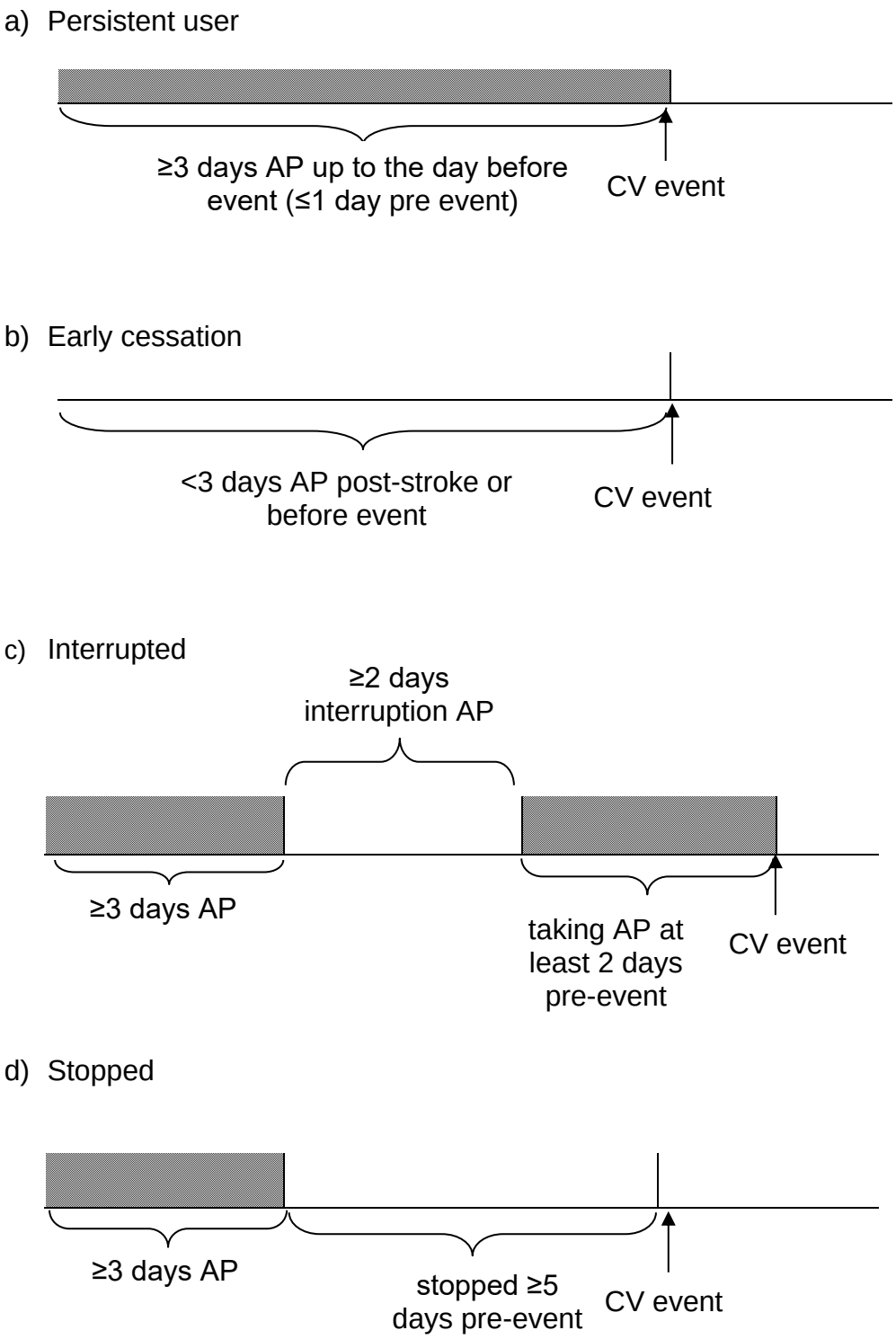


Figure 5-3: Determination of persistent user (a), early cessation user (b), interrupted user (c) and stopped user (d) of antiplatelet exposure.

AP=antiplatelet, CV=cardiovascular event.

5.2.9 Statistical analysis

Baseline characteristics of study participants including demographics, medical history data, antiplatelet regimen and category of antiplatelet exposures were summarised for cases and controls separately. For categorical variables, they were summarised using frequencies and percentages. Whereas for continuous variables, they were summarised using mean [standard deviation (SD)] or median [interquartile range (IQR)]. Baseline characteristics of study participants and bleeding events [intracranial haemorrhage (ICH) and extracranial haemorrhage (ECH)] were also summarised according to the three types of antiplatelet exposures.

To account for the individual matching, conditional logistic regression was performed to estimate odds ratio (OR) and 95% confidence intervals (95% CI) for cardiovascular event comparing patients who had early cessation, stopped/ interrupted and persistent (the reference group) of antiplatelet. First, univariable analysis was conducted to estimate crude effects for all variables, including antiplatelet exposure. Second, all variables were assessed in a more complex, multivariable model. In multivariable analysis, first, all variables were included (first model). Then, the least significant variable was consecutively dropped until all included variables were significant at $p<0.05$ (final model). A $p<0.05$ was considered significant. Point estimates OR and 95% CI are presented for all results.

SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) was used to find matched controls and the rest of analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp, 2012).

5.3 Results

5.3.1 Baseline characteristics

Complete data were available for analysis of antiplatelet exposure in 4050 patients. There were 239 cardiovascular events following an acute stroke. Forty-five of these events were excluded because they occurred within 2 days following acute stroke. The remaining 194 case patients were matched with 776 controls from the cohort. Table 5-1 shows the incidence of cardiovascular events following acute stroke that has become cases. The incidence of recurrent ischaemic stroke was higher than acute coronary syndrome and transient ischaemic attack.

Table 5-1: Incidence of cardiovascular events following acute ischaemic stroke.

Cardiovascular event	Number of events	Incidence (%)
Recurrent stroke	126	13.0
Acute coronary syndrome	45	4.6
Transient ischaemic attack	23	2.4

Table 5-2 summarizes the demographic and medical history characteristics of cases and controls. Cases and controls were of similar age at the diagnosis of acute ischaemic stroke (median 73 years for cases; 73 years for controls) and shared similar proportion of male sex (52.6%). Cases were significantly more likely than controls to have diabetes (31.4% versus 21.5%, p=0.003), a history of heart failure (10.9% versus 6.7%, p=0.046) or previous TIA (10.8% versus 6.9%, p=0.049). Approximately one fifth of cases and controls had previous stroke prior to their acute event.

Table 5-2: Characteristics of study participants

Characteristics	Cases n=194	Controls n=776	OR (95% CI)	p-value
Age, years				
Mean (SD)	70.90 (11.303)	70.97 (10.676)	-	-
Median (IQR)	73.00 (64-79)	73.00 (66-79)		
Male sex	102 (52.6)	408 (52.6)	-	-
Caucasian	161/187 (86.1)	647/747 (86.6)	0.94 (0.58-1.51)	0.782
Current Smoker	60/187 (32.1)	207/749 (27.6)	1.25 (0.87-1.79)	0.219
Baseline NIHSS†	11.5 (8-17)	12 (8-17)	0.99 (0.97-1.02)	0.886
Medical history				
Hypertension‡	147/194 (75.8)	545/753 (72.4)	1.19 (0.82-1.72)	0.356
Diabetes‡	61/194 (31.4)	167/776 (21.5)	1.71 (1.20-2.46)	0.003
Atrial fibrillation‡	37/194 (19.1)	122/753 (16.2)	1.27 (0.82-1.95)	0.285
Heart failure‡	20/184 (10.9)	45/675 (6.7)	1.80 (1.01-3.20)	0.046
Ischaemic heart disease‡	57/187 (30.5)	186/728 (25.5)	1.26 (0.89-1.80)	0.208
Previous TIA‡	19/176 (10.8)	50/725 (6.9)	1.78 (1.01-3.15)	0.049
Previous stroke‡	40/186 (21.5)	137/700 (19.6)	1.08 (0.72-1.62)	0.700
rt-PA	70 (36.1)	248 (32.0)	1.20 (0.87-1.66)	0.267

All values are reported as no. (%) unless otherwise noted. *Values are reported as mean (SD); †median (IQR); ‡missing some data at baseline. CI=confidence interval, IQR=interquartile range, NIHSS=National Institute Health Stroke Scale, OR=odds ratio, TIA=transient ischaemic attack, rt-PA=recombinant tissue plasminogen activator, SD=standard deviation.

5.3.2 Distribution of antiplatelet regimen and antiplatelet exposures

Antiplatelet regimen prescribed for cases and controls is shown in Table 5-3. Within 90 days following acute stroke, aspirin, clopidogrel, aspirin plus clopidogrel, aspirin plus dipyridamole and ticlopidine were the five commonest antiplatelet regimen prescribed. Within 90 days after acute stroke onset, 139 (71.6%) cases and 566 (72.9%) controls had been prescribed with a single antiplatelet regimen i.e. aspirin. Approximately one seventh of cases and controls were prescribed with clopidogrel after their acute event. About 3 to 5% of cases and controls were prescribed with DAPT, either aspirin plus clopidogrel or dipyridamole.

Table 5-3: Characteristics of antiplatelet regimen prescribed

Antiplatelet regimen	Cases n=194	Controls n=776	Odds ratio (95% CI)	p-value
Aspirin	139 (71.6)	566 (72.9)	1.00	-
Clopidogrel	28 (14.4)	105 (13.5)	1.10 (0.69-1.74)	0.674
Aspirin+ Clopidogrel	10 (5.2)	25 (3.2)	1.63 (0.75-3.52)	0.215
Aspirin+ Dipyridamole	10 (5.2)	50 (6.4)	0.82 (0.40-1.68)	0.585
Ticlopidine	4 (2.1)	16 (2.1)	1.01 (0.34-3.05)	0.980
Aspirin+ Ticlopidine	1 (0.5)	1 (0.1)	4.44 (0.28-71.29)	0.293
Carbasalate	1 (0.5)	5 (0.6)	0.76 (0.09-6.73)	0.825
Dipyridamole	1 (0.5)	5 (0.6)	0.79 (0.09-6.73)	0.825
Ozagrel	0 (0.0)	1 (0.1)	-	-
Triflusal	0 (0.0)	2 (0.3)	-	-

All values are reported as no. (%) unless otherwise noted. CI=confidence interval, OR=odds ratio.

While the majority of cases and controls were prescribed with aspirin, a few had aspirin stopped or interrupted and around 20% of cases and controls had early cessation of aspirin. The frequency distribution of antiplatelet exposure among cases and controls is shown in Table 5-4. Overall, more than 50% cases and controls had persistent antiplatelet exposure with clopidogrel, aspirin plus clopidogrel, aspirin plus dipyridamole or ticlopidine.

Table 5-4: Frequency of antiplatelet exposures according to five commonest antiplatelet regimen

Antiplatelet regimen	Cases n=194	Controls n=776
Aspirin		
Early cessation	27 (19.4)	133 (23.5)
Stopped/Interrupted	8 (5.8)	45 (8.0)
Persistent	104 (74.8)	388 (68.6)
Clopidogrel		
Early cessation	9 (32.1)	29 (27.6)
Stopped/Interrupted	2 (7.1)	5 (4.8)
Persistent	17 (60.7)	71 (67.6)
Aspirin+Clopidogrel		
Early cessation	5 (50.0)	7 (28.0)
Stopped/Interrupted	0 (0)	3 (12.0)
Persistent	5 (50.0)	15 (60.0)
Aspirin+Dipyridamole		
Early cessation	5 (50.0)	8 (16.0)
Stopped/Interrupted	0 (0)	4 (8.0)
Persistent	5 (50.0)	38 (76.0)
Ticlopidine		
Early cessation	2 (50.0)	5 (31.3)
Stopped/Interrupted	0 (0)	0 (0)
Persistent	2 (50.0)	11 (68.8)

All values are reported as no. (%) unless otherwise noted.

The demographic and medical history characteristics according to the types of antiplatelet exposures are summarized in Table 5-5. Persistent users and interrupted/stopped users had similar age and slightly younger than early cessation users at the diagnosis of acute stroke (median 72 years for persistent; 75.5 years for early cessation; 71.5 years for interrupted/stopped). At the baseline, interrupted/stopped users had more severity of stroke symptoms (median NIHSS 17) than the other two groups (median NIHSS 11 for persistent; NIHSS 13 for early cessation). Early cessation users had a more frequent history of atrial fibrillation, heart failure or ischaemic heart disease compared to others. However, history of previous stroke of interrupted/stopped users were more frequently than persistent and early cessation users (29% versus 18% versus 23.3%), whereas the frequency of previous TIA was similar among three groups.

Table 5-5: Characteristics of study participants according to types of antiplatelet exposure

Characteristics	Persistent user n=670	Early cessation user n=232	Interrupted/ Stopped user n=68	Overall n=970
Age, years				
Mean (SD)	70.23 (10.869)	73.62 (10.019)	69.12 (11.296)	70.96 (10.798)
Median (IQR)	72 (64-78)	75.5 (69-81)	71.5 (63.3-77)	73 (65-79)
Male sex	351 (52.4)	122 (52.6)	37 (54.4)	510 (52.6)
Ethnicity, Caucasian	556/646 (86.1)	197/222 (88.7)	55/66 (83.3)	808/934 (86.5)
Current Smoker	185/643 (28.8)	57/226 (25.2)	25/67 (37.3)	267/936 (28.5)
Baseline NIHSS†	11 (8-16)	13 (9-17)	17 (12-20)	12 (8-17)
Medical history				
Hypertension‡	473/654 (72.3)	169/225 (75.1)	50/68 (73.5)	692/947 (73.1)
Diabetes‡	164/670 (24.5)	51/232 (22.0)	13/68 (19.1)	228/970 (23.5)
Atrial fibrillation‡	91/654 (13.9)	56/225 (24.9)	12/68 (17.6)	159/947 (16.8)
Heart failure‡	38/601 (6.3)	21/196 (10.7)	6/62 (9.7)	65/859 (7.6)
Ischaemic heart disease‡	146/628 (23.2)	74/220 (33.6)	23/67 (34.3)	243/915 (26.6)
Previous TIA‡	48/623 (7.7)	16/212 (7.5)	5/66 (7.6)	69/901 (7.7)
Previous stroke‡	111/617 (18.0)	48/207 (23.2)	18/62 (29.0)	177/886 (20.0)
rt-PA	224 (33.4)	74 (31.9)	20 (29.4)	318 (32.8)

All values are reported as no. (%) unless otherwise noted. *Values are reported as mean (SD); †median (IQR); ‡missing some data at baseline. NIHSS=National Institute Health Stroke Scale, TIA=transient ischaemic attack, rt-PA=recombinant tissue plasminogen activator, SD=standard deviation, IQR=interquartile range.

The distribution of the antiplatelet regimen over the types of antiplatelet exposures is presented in Table 5-6. As shown, the majority of antiplatelet users were prescribed with aspirin. Following acute stroke, early cessation users were prescribed with clopidogrel more frequently than persistent and stopped/interrupted users (16.4% versus 13.1% versus 10.3%). Combination of two antiplatelet agents were prescribed fairly evenly across the three groups. The remaining, 4% persistent, 3.9% early cessation and 1.5% interrupted/stopped users were prescribed with ticlopidine, aspirin plus ticlopidine, carbasalate, dipyridamole, ozagrel and triflusal.

Table 5-6: Frequency of antiplatelet regimen according to types of antiplatelet exposure

Antiplatelet regimen	Persistent user n=670	Early cessation user n=232	Interrupted/ Stopped user n=68	Overall n=970
Aspirin	492 (73.4)	160 (69.0)	53 (77.9)	705 (72.7)
Clopidogrel	88 (13.1)	38 (16.4)	7 (10.3)	133 (13.7)
Aspirin/Clopidogrel	20 (3.0)	12 (5.2)	3 (4.4)	35 (3.6)
Aspirin/Dipyridamole	43 (6.4)	13 (5.6)	4 (5.9)	60 (6.2)
Ticlopidine	13 (1.9)	7 (3.0)	0 (0.0)	20 (2.1)
Aspirin/Ticlopidine	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.2)
Carbasalate	6 (0.9)	0 (0.0)	0 (0.0)	6 (0.6)
Dipyridamole	4 (0.6)	2 (0.9)	0 (0.0)	6 (0.6)
Ozagrel	0 (0.0)	0 (0.0)	1 (1.5)	1 (0.1)
Triflusal	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.2)

All values are reported as no. (%) unless otherwise noted.

As shown in Table 5-7, the occurrence of bleeding events was higher in early cessation users (5.2%) followed by interrupted/stopped users (1.5%). The total bleeding events were spread fairly between ICH and ECH. Similarly, early cessation users had the highest event of ICH and ECH compared to the other two groups.

Table 5-7: Frequency of bleeding events following antiplatelet exposure

Bleeding	Persistent user n=670	Early cessation user n=232	Interrupted/ Stopped user n=68	Overall n=970
ICH	3 (0.4)	7 (3.0)	1 (1.5)	11 (1.1)
ECH	6 (0.9)	6 (2.6)	0 (0.0)	12 (1.2)
Total bleeding	9 (1.3)	12 (5.2)	1 (1.5)	22 (2.3)

All values are reported as no. (%) unless otherwise noted. ICH=intracranial haemorrhage, ECH=extracranial haemorrhage.

5.3.3 Antiplatelet exposure and cardiovascular event

The main analysis assessed the effects of persistent, early cessation and interrupted/stopped antiplatelet users prescribing at any time following acute ischaemic stroke prior to index date, on the risk of cardiovascular event. Unadjusted and adjusted OR for the association between cardiovascular event and the types of antiplatelet exposures of interest are summarized in Table 5-8 and Table 5-9.

Univariable analysis

Compared to persistent users, early cessation users and interrupted/stopped users had a similar risk of cardiovascular event (OR,1.07; 95% CI, 0.67-1.71; p=0.784 and OR, 0.67; 95% CI, 0.34-1.36; p=0.269 respectively) (Table 5-8).

Table 5-8: Univariate analyses (conditional logistic regression) of antiplatelet exposures against outcome of being "case".

Antiplatelet exposures	Cases	Controls	OR (95% CI)	p-value
Early cessation	48 (24.7)	184 (23.7)	1.07 (0.67-1.71)	0.784
Stopped/Interrupted	10 (5.2)	58 (7.5)	0.67 (0.34-1.36)	0.269
Persistent	136 (70.1)	534 (68.8)	1.00	

All values are reported as no. (%) unless otherwise noted. CI=confidence interval, OR=odds ratio.

Multivariable analysis

No association was observed between early cessation and cardiovascular event when compared with persistent users after adjusting for diabetes and previous TIA (adjusted OR, 1.04; 95% CI, 0.62-1.74; p=0.876) (Table 5-9). Similarly, after adjustment with similar variables, interrupted/stopped users did not have a higher risk compared to persistent users (adjusted OR, 0.70; 95% CI, 0.33-1.48; p=0.352).

Table 5-9: Multivariable conditional logistic regression of explanatory variables against outcome of being "case"

Characteristics	Adjusted odds ratio (95% CI)	p-value*
First model, all variables		
Caucasian	0.89 (0.52-1.54)	0.684
Current Smoker	1.18 (0.77-1.81)	0.442
Baseline NIHSS	0.98 (0.95-1.02)	0.331
Hypertension	1.04 (0.65-1.67)	0.862
Diabetes	1.60 (1.03-2.49)	0.036
Atrial fibrillation	1.32 (0.76-2.29)	0.318
Heart failure	1.33 (0.69-2.55)	0.398
Ischaemic heart disease	0.99 (0.65-1.50)	0.964
Previous TIA	2.15 (1.15-4.01)	0.016
Previous stroke	0.97 (0.59-1.59)	0.896
rt-PA	1.05 (0.72-1.54)	0.787
Early cessation†	1.09 (0.60-1.96)	0.779
Stopped/Interrupted†	0.72 (0.32-1.65)	0.441
Final model		
Diabetes	1.72 (1.17-2.52)	0.006
Previous TIA	1.90 (1.06-3.40)	0.031
Early cessation AP†	1.04 (0.62-1.74)	0.876
Stopped/Interrupted AP†	0.70 (0.33-1.48)	0.352

*Adjusted for other variables in model. †Compared to Persistent users. AP=antiplatelet, CI=confidence interval, NIHSS=National Institutes of Health Stroke Scale, rt-PA=recombinant tissue plasminogen activator, TIA=transient ischaemic attack.

5.4 Discussion

In an effort to elucidate the rate of stopping or interruption antiplatelet in acute ischaemic stroke, a nested case-control study was performed and assessed the types of antiplatelet exposure and its association with cardiovascular event in 194 cases and 776 controls of acute ischaemic stroke. The present study found no evidence for an increased risk of cardiovascular event among patients who had early cessation or interrupted/stopped antiplatelet therapy within 90 days following acute ischaemic stroke.

There was no significant association with early cessation or interrupted/stopped antiplatelet therapy and the risk of cardiovascular event in the present study, which was inconsistent with previous studies. Studies by Maulaz, et al. (2005), Broderick, et al. (2011) and García Rodríguez, et al. (2011) found that patients who discontinued antiplatelet within one to six months is associated with an increased risk of stroke. The differences may be due to the small number of patients included in the current study compared with studies by Broderick, et al. (2011) and García Rodríguez, et al. (2011). In addition, there were differences in terms of duration of follow-up between the present and previous studies. For example, the study cohort in García Rodríguez, et al. (2011) was followed up at approximately 3.4 years which allow more number of events to be captured. On the other hand, similar findings were reported from Mehran, et al. (2013) and Ferreira-Gonzalez, et al. (2012) who also did not detect any associations between antiplatelet interruption and cardiovascular events. Although the finding in the present study was consistent in all studies, the interruption rates and subsequent outcomes were low in these cohorts and thus may not be able to detect small differences.

Antiplatelet therapy is indicated following ACS and acute ischaemic stroke in order to reduce recurrence event. Randomized clinical trials by Altman, et al. (1994), Diener, et al. (1996) and Gent, et al. (1996) have demonstrated that antiplatelet therapy is valuable after an acute ischaemic stroke and acute coronary syndrome. Aspirin has been shown to reduce the risk of recurrence stroke by 15% (Johnson, et al., 1999), whereas clopidogrel and combination of aspirin/dipyridamole have shown a similar efficacy in prevention of second strokes (Sacco, et al., 2008).

According to NICE guidelines for management of ischaemic stroke, antiplatelet therapy should be continued for a long term (National Institute for Health and Clinical Excellence, 2008) and a study by Biondi-Zoccai, et al. (2006) recommends that antiplatelet therapy should never be interrupted for patients with cardiovascular disease. Under certain circumstances, there are reasons to stop or interrupt antiplatelet therapy, especially when there is associated with adverse events such as bleeding or allergic reactions; or interrupted by physician to reduce the risk of bleeding during procedures. Any reasons to stop or interrupt antiplatelet therapy, could expose patients to an increased risk of thrombotic event.

The absence of an association with early cessation or interrupted/stopped antiplatelet therapy with the risk of cardiovascular event in the present study is warrant further investigation. It is important to conduct this study looking for an association of antiplatelet exposure as the risk of recurrent stroke is greatest during the first three months after stroke. Although this study found no evidence for an increased risk of cardiovascular event among patients who interrupted/stopped and early cessation antiplatelet, the occurrence of bleeding events was found higher in those who took antiplatelet less than three days (early cessation users) prior to cardiovascular event. However, other than bleeding events, other reasons for interruption and stopped antiplatelet could not be captured in this study.

5.4.1 Strengths and limitations

A major strength of the present study is the use of VISTA database sample which provided data that were prospectively collected during clinical trials in patients with confirmed ischaemic stroke. This cohort of ischaemic stroke patient from which all cases of cardiovascular event following ischaemic stroke and a random sample of controls without cardiovascular event were selected. The nested case-control study design is useful to determine if a drug exposure is associated with an outcome. This study design avoided the common problem of selection bias which is typical in many case-control studies. Selection bias was minimised by including all cases of cardiovascular event within the study cohort and matched controls were drawn from the same population as the cases and selected

independently of their exposure status. Cases were matched up to four controls could increase the precision of the study findings. Matching by age and sex could increase the study efficiency in the present study and helped ensure that cases and controls were comparable. Rose and van der Laan (2009) has also described that matching between cases and controls according to certain characteristics able to eliminate confounding and to gain in efficiency.

As with all observational studies, this study has limitations. One reason why this study did not show a significant difference to assess the association of cardiovascular event and antiplatelet exposures due to small numbers of patients included in this study. Due to small sample size, the study had low power to detect associations, thus, the study findings should be interpreted bearing in mind the likelihood of type II error. On the other hand, due to lack of data on the cause of ischaemic stroke, dose of antiplatelet therapy, medication compliance and reasons for interrupted, stopped or early cessation of antiplatelet regimen, may have masked a true relationship between cardiovascular event and exposure of antiplatelet. Lastly, this study was based on data available in the stroke trial registry, thus only included patients who met the eligibility of inclusion criteria of clinical trial and excluded any individuals not participate in trials.

5.5 Conclusion

In patients who have suffered ischaemic stroke, this study found that early cessation or interrupted/stopped antiplatelet therapy was not associated with an increased risk of cardiovascular events compared to persistent users. This could be due to the sample size may be too small and has insufficient power to detect this effect. Further research is needed to investigate reasons for antiplatelet interrupted and stopped and its association with the risk of recurrence ischaemic stroke.

6 Cardiovascular events after dual antiplatelet therapy cessation in patients with a history of an acute coronary syndrome

6.1 Introduction

Antiplatelet therapy is indicated after an acute coronary syndrome (ACS) because it lowers mortality and reduces the early risk of recurrence. Dual antiplatelet therapy (DAPT), typically using aspirin and an ADP receptor antagonist such as clopidogrel, ticagrelor or prasugrel, is superior to use of a single antiplatelet agent. Clopidogrel, when given together with aspirin and fibrinolytic therapy has been shown to reduce the rate of death or re-infarction without major increased bleeding risk following ST-elevation myocardial infarction (STEMI) when compared with aspirin alone (Chen, et al., 2005; Sabatine, et al., 2005b). Further, in the CURE trial, ACS patients were randomised to either clopidogrel or placebo in addition to aspirin for 3 to 12 months. The results showed a significant reduction in incidence of death from cardiovascular causes, non-fatal myocardial infarction or stroke in the clopidogrel treatment group (9.3 % compared to 11.4 % in placebo) (Yusuf, et al., 2001).

There are reports supported the cardiovascular risk is increased in the period following cessation of DAPT and initiation of monotherapy. Studies by Ho *et al*. found the first 90 days interval after clopidogrel cessation was associated with significantly higher risk of cardiovascular events compared with 91-180 days interval in both medical or PCI-treated patients (Ho, et al., 2008b; Ho, et al., 2010). There are suggestions that this event maybe due to a rebound in platelet activity (Lordkipanidze, et al., 2009; Sambu, et al., 2011a). It is clear that data show cessation of DAPT may put patients at risk and a better understanding of this may allow strategies to mitigate this risk.

The National Health Service Greater Glasgow and Clyde (NHSGGC) Safe Haven is a secure data repository consisting of Scottish Morbidity Record (SMR), General Register Office for Scotland (GROS) death registration and the Prescribing Information System (PIS) database. Thus, it allows identification of patients with

ACS, identification of any performed coronary interventions, information on prescription redemption, and follow-up of clinical outcomes. This data source was used to assess the incidence and predictors of cardiovascular event after discontinuation of DAPT in patients who have suffered ACS.

6.2 Methods

6.2.1 Study setting and study population

General details of the study setting and study population are described in section 2.2.2. Data were incorporated from few datasets, Scottish Morbidity Record, General Register Office for Scotland death registration, Prescribing Information System and Scottish Care Information – Diabetes Collaboration (SCI-DC) and General Practice database.

Patients were included in the study if they experienced an ACS event between 1 January 2008 and 31 December 2013, dispensed with DAPT, filled a prescription for DAPT within 60 days of hospital discharge and continued with single antiplatelet therapy after treatment of DAPT. An ACS event was defined as emergency hospital admission with a main discharge diagnosis of ACS (International Classification of Diseases [ICD-10] codes I20, I21 or I22).

6.2.2 Demographic data

For each patient, data on age, sex, hospital admissions and discharge dates, and operations were obtained from SMR01 dataset. Baseline demographic data such as smoking status, hypertension, hyperlipidaemia, renal disease and ischaemic heart disease were obtained from General Practice dataset, whereas diabetes data from the SCI-DC database. These comorbidities were defined as a condition reported from general practitioners before the ACS event or in any subsequent visit to general practitioner of ACS occurrence. Whereas, for a coronary revascularization procedure, it was defined as a procedure listed in operation field from SMR01 dataset during the index admission. Revascularization procedures include coronary artery bypass graft (CABG) (English Office of Population Censuses and

Surveys (OPCS-4) codes K40-46) and percutaneous transluminal coronary angioplasty (PTCA) (OPCS-4 codes K49-50 and K75).

6.2.3 Medication use

The usage of DAPT was identified through PIS data, which records the date dispensed and the number of days supplied for each dispensed medication. DAPT use included and combination of aspirin-clopidogrel, aspirin-dipyridamole, aspirin-ticagrelor and aspirin-prasugrel. The number of pills dispensed for each prescription was used to calculate the duration of DAPT use. Discontinuation of DAPT was defined when there is no refill prescription of one antiplatelet drug in the combination regime, or dispense date more than 14 days from the last DAPT refill date plus the number of days supplied for that last refill. The last day of DAPT use was based on the date of last prescription refills plus the number of days supplied for that refill.

6.2.4 Study endpoints

The primary endpoint of the study was a cardiovascular event, which consists of composite of all-cause mortality, ACS (myocardial infarction (MI) or unstable angina), transient ischaemic attack (TIA), stroke or heart failure after cessation of DAPT. Information on death was obtained through General Register Office for Scotland database. The ICD-10 codes were used to identified hospitalizations for ACS (I20 – I25), TIA (G458-G459), stroke (I63-I64) and heart failure (I50) (**Appendix 5**).

6.2.5 Statistical analysis

Descriptive statistics were recorded for recent ACS patients. A separate analysis of descriptive statistics was also recorded for patients during DAPT treatment and for those who were event-free at DAPT cessation, differentiating between patient with medical and revascularization therapy. Categorical variables were summarised using frequencies and proportions and continuous variables as mean [standard deviation (SD)] or median [interquartile range (IQR)]. Patients reached the end of the follow-up period without experiencing an endpoint were censored.

The life table method was used to calculate unadjusted incidence rates per 1000 patient-days of cardiovascular events for each 90-day interval during DAPT and after DAPT cessation and stratified by medical and revascularization treated patients. Incidence rate ratios (IRR) with 95% confidence interval (95% CI) was calculated to compare a difference between incidence rates for medical and revascularization-treated patients from the same interval.

Several analyses were then performed to assess the association between time interval after stopping DAPT and risk of cardiovascular events; and to determine predictors in cardiovascular event after stopping DAPT. First, Poisson regression was used to assess the association between time interval after stopping DAPT and risk of cardiovascular events, where incidence rate ratios was calculated and adjusting for age, medical or revascularization therapy and duration of DAPT. In these models, the risk of cardiovascular events in the first 90 days interval after stopping DAPT was compared with the 91 to 360 days interval and with the 91 to 180 days interval consistent with prior studies (Ho, et al., 2008b; Ho, et al., 2010). Second, Cox proportional hazards regression analysis was used to model the risk factors of cardiovascular event after stopping DAPT. Univariate Cox proportional hazards regression analysis was used to estimate the risk ratios for all factors under consideration. Then multivariate proportional hazards regression analysis was performed to identify the collection of risk factors making independent contributions to risk of cardiovascular event. With regard to statistical analysis, the models were adjusted for age, medical or revascularization therapy and duration of DAPT. Adjustment was not made for hypertension, diabetes, hyperlipidaemia, chronic renal failure and ischaemic heart disease because of missing data.

Sub-group analysis was performed for medical vs revascularization treated patients, duration of DAPT six months and less vs more than six months, and antiplatelet therapy used after DAPT cessation i.e. clopidogrel vs aspirin. The hazard ratios (HRs) for cardiovascular event were generated using Cox proportional hazard models as described in the earlier paragraph.

Statistical software IBM SPSS Statistics version 21.0 (IBM Corp, 2012) was used to perform all descriptive, survival analysis, the Poisson regression and the Cox proportional hazard regression, while StatsDirect Statistical Software version 3.0.171 was used to compare two incidence rates between medical and revascularization-treated patients. A value of $p<0.05$ was considered statistically significant.

6.3 Results

6.3.1 Patient selection

A total of 7232 patients admitted with ACS during the study period. Of these, 1872 patients refilled DAPT within 60 days of hospital discharge. Of 1872 patients, 1340 (71.6%) stopped DAPT and continued with single antiplatelet therapy (Figure 6-1). Among 1340 patients, 1129 (84.3%) were event-free at DAPT cessation.

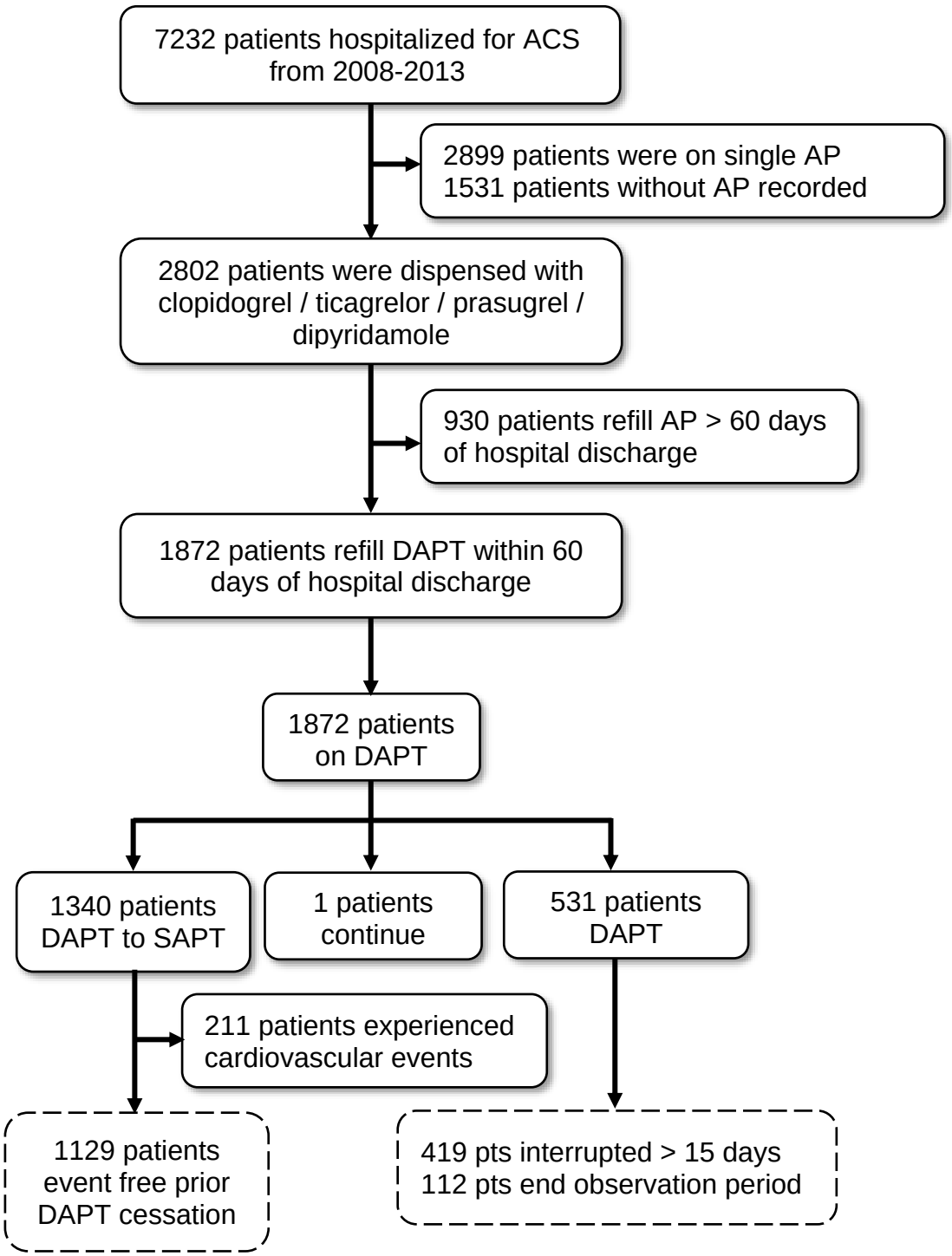


Figure 6-1: Flow diagram describing the selection of data from the NHS Scotland National safe haven for the analysis reported.

AP=antiplatelet, DAPT=dual antiplatelet therapy, SAPT=single antiplatelet therapy

6.3.2 Demographics of samples

Demographic characteristics for the population hospitalised with ACS, during DAPT and after DAPT cessation patients are displayed in Table 6-1. The mean (SD) age for ACS population was 68.98 (13.90) years, and 42.8% were female. On the other hand, the mean (SD) age for during DAPT sample was 64.94 (13.02) years, and 38.1% were female. As for after DAPT cessation sample, the mean (SD) age for was 64.55 (12.98) years, and 36.2% were female.

Table 6-1: Demographic characteristics

Characteristics	ACS patients (n=7232)	During DAPT (n=1340)	After DAPT cessation (n=1129)
Age, years			
Mean (SD)	68.96 (13.95)	64.94 (13.02)	64.55 (12.98)
Median (IQR)	70 (58-80)	64 (55-75)	64 (55-75)
Sex, n (%)			
Female	3098 (42.8)	510 (38.1)	409 (36.2)
Male	4134 (57.2)	830 (61.9)	720 (63.8)

SD=standard deviation, IQR=interquartile range

An additional analysis was performed to compare the study sample with the excluded sample (Table 6-2). There was a significant difference in the age at the time of ACS diagnosis between patients included in the study sample and those excluded from the analysis: the median age of the latter group was 71 years (IQR, 59-81 years) versus 64 years (IQR, 55-75 years) (p<0.0001). The proportion of female patients was significantly less in the study sample (38% vs 44%; p=0.000).

Table 6-2: Comparison of the study sample with the sample excluded from analysis

Characteristics	Patients included in study sample (n=1340)	Patients excluded from analysis (n=5892)	p-value
Age, years			
Mean (SD)	64.94 (13.02)	69.88 (13.99)	<0.0001*
Median (IQR)	64 (55-75)	71 (59-81)	
Sex, n (%)			
Female	510 (38.1)	2588 (43.9)	0.000†
Male	830 (61.9)	3304 (56.1)	

*Statistical analysis of the difference in mean age (Mann-Whitney U test). †Statistical analysis (X^2 test). SD=standard deviation, IQR=interquartile range

6.3.3 Baseline characteristics of study samples

Baseline characteristics of during DAPT and after DAPT cessation patients are shown in Table 6-3. Among the 1340 patients while on DAPT, 28.3% were current smokers, 34.6% had hypertension, 17.5% had diabetes, 64.7% had hyperlipidaemia, 14.5% had chronic renal failure and 24.1% had ischaemic heart disease. All patients were treated with aspirin and more than 90% combined with clopidogrel (94.2%) followed by ticagrelor (3.1%), dipyridamole (2.4%) and prasugrel (0.2%).

The 1129 patients after DAPT cessation, were predominantly male (63.8%) with a mean age (SD) of 64.55 (12.98) years. Cardiac risk factors included current smoking (28.3%), hypertension (35.2%), diabetes (17.8%), hyperlipidaemia (65.6%), chronic renal failure (15.1%) and ischaemic heart disease (25.0%). Thirty-two percent patients in this group were on prior antiplatelet. All patients in this study sample were on aspirin and in combination with clopidogrel (94.4%), ticagrelor (3.1%), dipyridamole (2.1%) and prasugrel (0.3%). Mean total duration (SD) of DAPT therapy following ACS was 176.64 (154.48) days. After DAPT cessation, mono antiplatelet therapy aspirin was commonly prescribed (79.1%) than clopidogrel (19.8%), followed by dipyridamole (0.6%) and ticagrelor (0.4%).

Table 6-3: Baseline characteristics

Characteristics	During DAPT			After DAPT cessation		
	All patients (n=1340)	Medical treated (n=933)	Revascularization treated (n=407)	All patients (n=1129)	Medical treated (n=754)	Revascularization treated (n=375)
Age, years						
Mean (SD)	64.94 (13.02)	66.68 (13.12)	60.94 (11.86)	64.55 (12.98)	66.44 (13.28)	60.75 (11.44)
Median (IQR)	64(55-75)	67(57-77)	60(52-69)	64(55-75)	67(57-77)	60(52-68)
Sex						
Female	510(38.1)	377(40.4)	133(32.7)	409(36.2)	291(38.6)	118(31.5)
Male	830(61.9)	556(59.6)	274(67.3)	720(63.8)	463(61.4)	257(68.5)
Current smoker	296/1047 (28.3)	178/694 (25.6)	118/353 (33.4)	259/914 (28.3)	152/580 (26.2)	107/334 (32.0)
Medical history						
Hypertension	463(34.6)	336(36.0)	127(31.2)	397(35.2)	276(36.6)	121(32.3)
Diabetes	234(17.5)	157(16.8)	77(18.9)	201(17.8)	129(17.1)	72(19.2)
Hyperlipidaemia	867(64.7)	591(63.3)	276(67.8)	741(65.6)	486(64.5)	255(68.0)
CRF	194(14.5)	140(15.0)	54(13.3)	170(15.1)	118(15.6)	52(13.9)
IHD	323(24.1)	218(23.4)	105(25.8)	282(25.0)	186(27.7)	96(25.6)
Prior antiplatelet therapy						
No prior AP	885(66.0)	576(61.7)	309(75.9)	770(68.2)	483(64.1)	287(76.5)
Aspirin	316(23.6)	234(25.1)	82(20.1)	253(22.4)	177(23.5)	76(20.3)
Clopidogrel	35(2.6)	29(3.1)	6(1.5)	24(2.1)	19(2.5)	5(1.3)
Dipyridamole	6(0.4)	6(0.6)	0(0)	5(0.4)	5(0.7)	0(0)
Aspirin + clopidogrel	59(4.4)	52(5.6)	7(1.7)	50(4.4)	44(5.8)	6(1.6)
Aspirin + dipyridamole	33(2.5)	30(3.2)	3(0.7)	24(2.1)	23(3.1)	1(0.3)
Aspirin + ticagrelor	5(0.4)	5(0.5)	0(0)	3(0.3)	3(0.4)	0(0)
DAPT combinations post-ACS						
Aspirin + clopidogrel	1261(94.2)	861(92.3)	400(98.3)	1066(94.4)	697(92.4)	369(98.3)
Aspirin + dipyridamole	33(2.4)	30(3.2)	3(0.7)	24(2.1)	22(2.9)	2(0.6)
Aspirin + ticagrelor	42(3.1)	38(4.0)	4(1.0)	35(3.1)	31(4.1)	4(1.1)
Aspirin + prasugrel	3(0.2)	3(0.3)	0(0)	3(0.3)	3(0.4)	0(0)
Duration of DAPT						
Mean (SD)	175.09 (155.30)	164.28 (148.17)	199.85 (168.10)	176.64 (154.48)	163.02 (143.03)	204.03 (172.22)
Median (IQR)	113 (78-208)	109 (76-185)	126 (85-311)	112 (78-221)	108 (75-187)	128 (85-317)
≤ 6 months	955(71.3)	695(74.5)	260(63.9)	793(70.2)	558(74.0)	235(62.7)
> 6 months	385(28.7)	238(25.5)	147(36.1)	336(29.8)	196(26.0)	140(37.3)
≤ 12 months	1139(85.0)	812(87.0)	327(80.3)	948(84.0)	651(86.3)	297(79.2)
> 12 months	201(15.0)	121(13.0)	80(19.7)	181(16.0)	103(13.7)	78(20.8)
Follow-up duration after stopping DAPT, days						
Mean (SD)	-	-	-	1009.34 (534.79)	950.28 (553.12)	1126.97 (475.55)
Median (IQR)	-	-	-	1067 (615-1394)	982 (509-1364)	1155 (828-1463)
Single AP after DAPT cessation						
Aspirin	-	-	-	893(79.1)	596(79.0)	297(79.2)
Clopidogrel	-	-	-	224(19.8)	146(19.4)	78(20.8)
Dipyridamole	-	-	-	7(0.6)	7(0.9)	0(0)
Ticagrelor	-	-	-	5(0.4)	5(0.7)	0(0)

All values are reported as no. (%) unless otherwise noted. AP=antiplatelet, CRF=chronic renal failure, DAPT=dual antiplatelet therapy, IHD=ischaemic heart disease, SD=standard deviation, IQR=interquartile range.

6.3.4 Study endpoints

Of 1340 patients, while on DAPT, incidence of cardiovascular events occurred in 211 (15.7%) patients. Cardiovascular events occurred in 179 (19.2%) medical treated patients and 32 (7.9%) revascularization treated patients. Of 1129 patients after DAPT cessation, cardiovascular events occurred in 188 (16.7%) patients, which 154 (20.4%) occurred in medical and 34 (9.1%) in revascularization therapy (Table 6-4).

Table 6-4: Cardiovascular events during DAPT and after DAPT cessation

	During DAPT (n=1340)			After DAPT cessation (n=1129)		
	All	Medical treated (n=933)	Revascularization treated (n=407)	All	Medical treated (n=754)	Revascularization treated (n=375)
Cardiovascular events	211 (15.7)	179 (19.2)	32 (7.9)	188 (16.7)	154 (20.4)	34 (9.1)
ACS	179 (13.4)	155 (16.6)	24 (5.9)	60 (5.3)	46 (6.1)	14 (3.7)
Ischaemic stroke/TIA	2 (0.1)	2 (0.2)	0 (0)	24 (2.1)	16 (2.1)	8 (2.1)
Heart failure	24 (1.8)	19 (2.0)	5 (1.2)	9 (0.8)	8 (1.1)	1 (0.3)
All death	44 (3.3)	35 (3.8)	9 (2.2)	135 (12.0)	120 (15.9)	15 (4.0)

All values are reported as no. (%) unless otherwise noted. ACS=acute coronary syndrome, DAPT=dual antiplatelet therapy, TIA=transient ischaemic attack.

6.3.5 Incidence rates of cardiovascular event

The incidence rates of cardiovascular event during DAPT and after DAPT cessation are presented in Table 6-5 and Figure 6-2 for each 90-day interval. While on DAPT, the incidence rate was higher in 0-90-day interval than in the next 90-day interval, 1.88 and 0.33 per 1000 patient-days, respectively. After cessation of DAPT, the incidence rate was lower in 0-90-day interval than in the next 90-day interval, 0.15 and 0.23 per 1000 patient-days, respectively.

Table 6-5: The risk of cardiovascular events following ACS while on DAPT and up to two years after DAPT cessation

Period, days	Number at risk	Number of events	Incidence rate per 1000 patient-days
During DAPT			
0-90	1340	181	1.882
91-180	797	17	0.328
181-270	354	5	0.184
271-360	250	3	0.152
361-450	189	1	0.088
451-540	64	1	0.231
541-630	32	1	0.453
631-720	17	0	0.000
After DAPT cessation			
0-90	1129	15	0.151
91-180	1072	22	0.234
181-270	1017	21	0.234
271-360	978	10	0.115
361-450	950	17	0.203
451-540	910	16	0.119
541-630	876	17	0.220
631-720	840	15	0.205

ACS=acute coronary syndrome, DAPT=dual antiplatelet therapy.

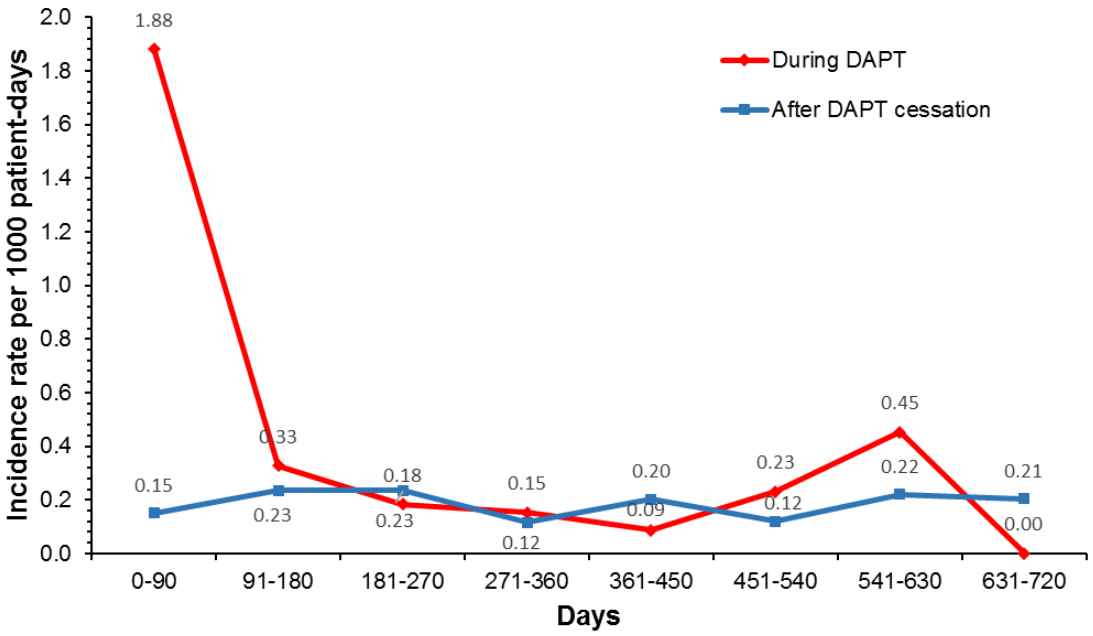


Figure 6-2: Incidence rate during and after DAPT cessation

Figure 6-3 shows the incidence rate for both medically and revascularization-treated patients while on DAPT and after DAPT cessation. The incidence rate for the first 90-day interval was higher in medically-treated patients than revascularization-treated patients, 2.34 and 0.91 per 1000 patient-days, respectively. Compared with revascularization-treated patients, medically-treated patients were associated with significantly increased risk of cardiovascular event for 0-90-day interval (IRR, 2.57; 95% CI, 1.71-3.99; p<0.0001) (Figure 6-3(a)).

After DAPT discontinuation, in the first 90-day interval, there was no difference in the incidence rate of cardiovascular event between medically-treated patients and revascularization-treated patients (IRR, 3.30; 95% CI, 0.75-30.12; p=0.096) (Figure 6-3(b) and Table 6-6). However, there were significantly increased risk of cardiovascular event for 91-180 days and 181-270 days in medically-treated patients (IRR, 5.16; 95% CI, 1.25-5.54; p=0.014 and IRR, 3.31; 95% CI, 0.97-17.54; p=0.042 respectively).

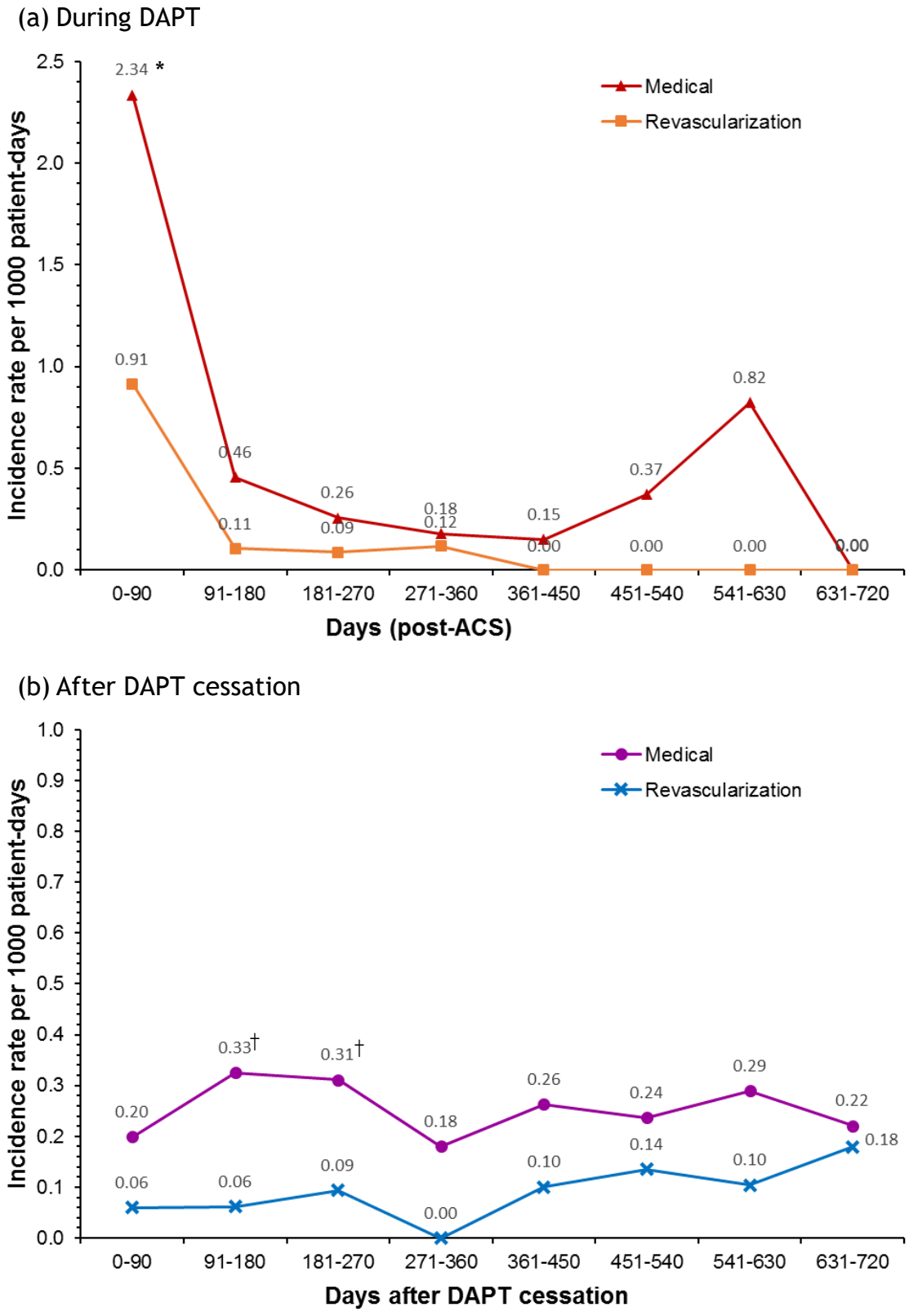


Figure 6-3: Incidence rate (a) during DAPT and (b) after DAPT cessation for medically- and revascularization-treated patients

*p<0.01, †p<0.05, compared with revascularization-treated patients from the same interval.

Table 6-6: Incidence rate of cardiovascular events after DAPT cessation for medical and revascularization treated patients

Period, days	Medically-treated			Revascularization-treated			Incidence rate ratio* (95% CI)	p-value
	Number at risk	Number of events	Incidence rate per 1000 patient-days	Number at risk	Number of events	Incidence rate per 1000 patient-days		
0-90	754	13	0.199	375	2	0.060	3.30 (0.75-30.12)	0.096
91-180	706	20	0.325	366	2	0.062	5.16 (1.25-5.54)	0.014
181-270	660	18	0.311	357	3	0.094	3.31 (0.97-17.54)	0.042
271-360	628	10	0.180	350	0	0.000	-	-
361-450	605	14	0.263	345	3	0.100	2.64 (0.74-14.34)	0.112
451-540	577	12	0.237	333	4	0.135	1.76 (0.53-7.51)	0.319
541-630	550	14	0.290	326	3	0.104	2.82 (0.79-15.30)	0.089
631-720	522	10	0.221	318	5	0.179	1.24 (0.39-4.64)	0.689

*Incidence rate ratio compared a difference incidence rate between medical and revascularization-treated patients. CI=confidence interval.

The incidence rates of cardiovascular events following DAPT discontinuation for yearly interval are shown in Figure 6-4 and Table 6-7. The Incidence rate was higher in year two than in year one after DAPT cessation, 0.21 and 0.18 per 1000 patient-days, respectively. The rate was decreased over the next three years and similar pattern can be seen for medically-treated patients. At the same time, medically-treated patients experienced a higher incidence rate compared to revascularization-treated patients for one-, two- and three-year interval after discontinuation of DAPT (IRR, 4.62; 95% CI, 2.22-11.98; p<0.0001; IRR, 1.97; 95% CI, 1.09-3.78; p=0.019 and IRR 2.94; 95% CI, 1.09-9.91; p=0.022 respectively).

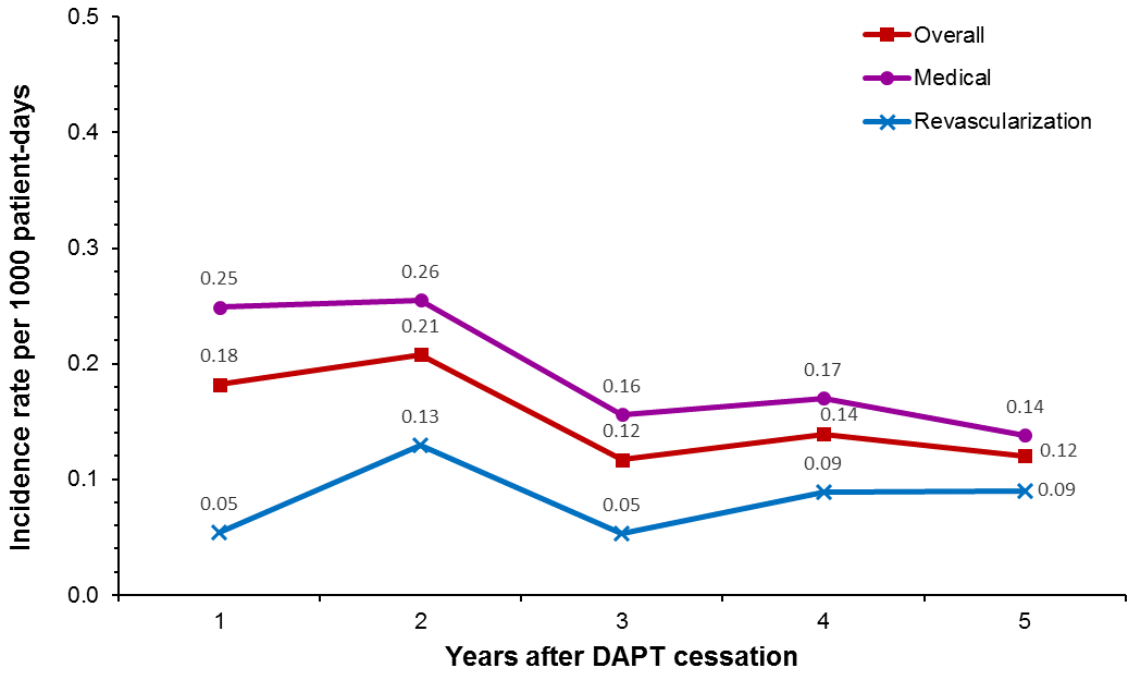


Figure 6-4: Incidence rate after DAPT cessation, stratified by medical and revascularization therapy

Table 6-7: Incidence rate of cardiovascular events yearly after DAPT cessation

Period, years	Number at risk	Number of events	Incidence rate per 1000 patient-days†	Medically-treated			Revascularization-treated			Incidence rate ratio* (95% CI)	p-value
				Number at risk	Number of events	Incidence rate per 1000 patient-days	Number at risk	Number of events	Incidence rate per 1000 patient-days		
1	1129	68	0.182	754	61	0.249	375	7	0.054	4.62 (2.22-11.98)	<0.0001
2	950	65	0.208	605	50	0.255	345	15	0.129	1.97 (1.09-3.78)	0.019
3	786	28	0.117	484	23	0.156	302	5	0.053	2.94 (1.09-9.91)	0.022
4	549	20	0.139	334	15	0.170	215	5	0.089	1.91 (0.66-6.71)	0.203
5	252	7	0.120	156	5	0.138	96	2	0.090	1.53 (0.25-16.04)	0.670

*Incidence rate ratio compared a difference incidence rate between medical and revascularization-treated patients. CI=confidence interval.

6.3.6 Adjusted incidence rate ratios

The adjusted IRR of cardiovascular event in the first 90 days compared, first, to the second 90 days (91-180-day) and second, to the 91-360-day interval after DAPT cessation are shown in Table 6-8. After adjusting for age, medical or revascularization therapy and total duration of DAPT treatment, there was no increased risk of cardiovascular event when compared the first 90 days with the 91-360-day interval (adjusted IRR, 0.59; 95% CI, 0.30-1.17; p=0.129). There were similar observations in medically and revascularization-treated group.

When compared with 91-180-day interval, after adjusting for age, medical or revascularization therapy and total duration of DAPT treatment, there was no increased risk of cardiovascular event the first 90 days (adjusted IRR, 0.70; 95% CI, 0.32-1.55; p=0.375). Similar observations were found in medical and revascularization therapy group.

Table 6-8: Adjusted incidence rate ratios for the cardiovascular event after DAPT cessation

Patient cohort	After DAPT cessation (n=1129)			Medical therapy (n=754)			Revascularization therapy (n=375)		
	n	Adjusted IRR (95% CI)	p-value	n	Adjusted IRR (95% CI)	p-value	n	Adjusted IRR (95% CI)	p-value
All patients*	1129	0.59 (0.30-1.17)	0.129	754	0.54 (0.26-1.13)	0.101	375	0.98 (0.16-6.04)	0.980
All patients†	1129	0.70 (0.32-1.55)	0.375	754	0.64 (0.28-1.50)	0.306	375	1.18 (0.13-10.96)	0.882

*comparing the initial 90-day interval versus the 91 to 360-day interval after stopping DAPT.
†comparing the initial 90-day interval versus the 91 to 180-day interval after stopping DAPT
All the Poisson regression models adjusted for age, medical or revascularization therapy and duration of DAPT. CI=confidence interval, IRR=incidence rate ratio.

6.3.7 Predictors of cardiovascular events after DAPT cessation

The univariate analysis of possible predictors of risk of cardiovascular events after stopping DAPT is presented in Table 6-9. Ten variables appeared to be significant predictors of cardiovascular events in the univariate analysis. The results of the final model of multivariate analysis are summarized in Table 6-10. The multivariate model indicated that increased age (HR, 1.07; 95% CI, 1.05-1.08; p=0.000) was significantly associated with an increased risk of cardiovascular event, whereas patients treated with revascularization (HR, 0.58; 95% CI, 0.39-0.85; p=0.005) and longer duration of DAPT (HR, 0.997; 95% CI, 0.995-0.998; p=0.000) were associated with a decreased risk.

6.3.8 Sub-group analysis

Sub-group analyses data are presented as a forest plot in Figure 6-5. Whereas, Figures 6-6 to 6-8 presents the survival curves showing the cumulative rates of cardiovascular events for the sub-group analyses.

a) Medically- and revascularization-treated patients

In unadjusted model, there is a decreased risk of cardiovascular events in revascularization-treated patients compared to medically treated (HR, 0.38; 95% CI, 0.26-0.55; p=0.000). The same is true in the model adjusting for age and duration of DAPT (HR, 0.58; 95% CI, 0.39-0.85; p=0.005). Figure 6-6 presents the survival curves among those with revascularization and those with medically treated.

b) DAPT duration

DAPT duration of ≤ 6 months was associated with an increased risk of cardiovascular event following discontinuation of DAPT, compared to those with DAPT duration more than 6 months (unadjusted HR, 2.23; 95% CI, 1.50-3.32; p=0.000). Even after adjustment, the difference in cardiovascular events risk between patients with DAPT duration ≤ 6 months and those with DAPT duration > 6 months remains statistically significant (adjusted HR, 1.51; 95% CI, 1.01-2.27; p=0.046). Figure 6-7(a) shows the survival curves between duration of DAPT ≤6 months vs >6 months.

Similarly, for DAPT duration ≤ 12 months, the risk of cardiovascular event was associated with increased risk compared to those with DAPT duration more than 12 months (unadjusted HR, 6.66; 95% CI, 2.74-16.20; p=0.000). After adjustment, the difference in cardiovascular event risk between those who received DAPT ≤ 12 months and >12 months patients remains statistically significant, with four times increase in risk of cardiovascular event among ≤ 12 months group as compared to >12 months group (adjusted HR, 4.14; 95% CI, 1.69-10.14; p=0.002). Figure 6-7(b) shows the survival curves between duration of DAPT ≤12 months vs >12 months.

c) Single antiplatelet therapy after DAPT cessation

In the unadjusted model, there is an increased risk of cardiovascular event in those who continued with clopidogrel as compared to those continued with aspirin (unadjusted HR, 1.40; 95% CI, 1.01-1.94; p=0.046). However, after adjustment for age, medical or revascularization therapy, and duration of DAPT, there is no statistically significant difference between clopidogrel and aspirin group in terms of cardiovascular event risk (adjusted HR, 1.33; 95% CI, 0.95-1.85; p=0.095). Figure 6-8 shows the survival curves between clopidogrel vs aspirin therapy.

Table 6-9: Univariate Cox proportional hazards regression analyses of possible predictors of cardiovascular events after DAPT cessation

Characteristics	HR (95% CI)	p-value
Age	1.08 (1.06-1.09)	0.000
Male	0.52 (0.39-0.69)	0.000
Current smoker	1.35 (0.69-2.66)	0.380
Hypertension	3.72 (2.44-5.66)	0.000
Diabetes	5.85 (2.75-12.45)	0.000
Hyperlipidaemia	1.56 (1.17-2.08)	0.002
Chronic renal failure	1.76 (1.08-2.85)	0.023
Ischaemic heart disease	5.34 (2.97-9.58)	0.000
Revascularization vs medical therapy	0.38 (0.26-0.55)	0.000
Prior vs no prior AP	2.02 (1.51-2.70)	0.000
DAPT duration	0.995 (0.994-0.997)	0.000

AP=antiplatelet, CI=confidence interval, DAPT=dual antiplatelet therapy, HR=hazard ratio.

Table 6-10: Final multivariate model of predictors of risk of cardiovascular events after stopping DAPT

Characteristics	HR (95% CI)	p-value
Age	1.07 (1.05-1.08)	0.000
Revascularization vs medical therapy	0.58 (0.39-0.85)	0.005
DAPT duration	0.997 (0.995-0.998)	0.000

CI=confidence interval, DAPT=dual antiplatelet therapy, HR=hazard ratio.

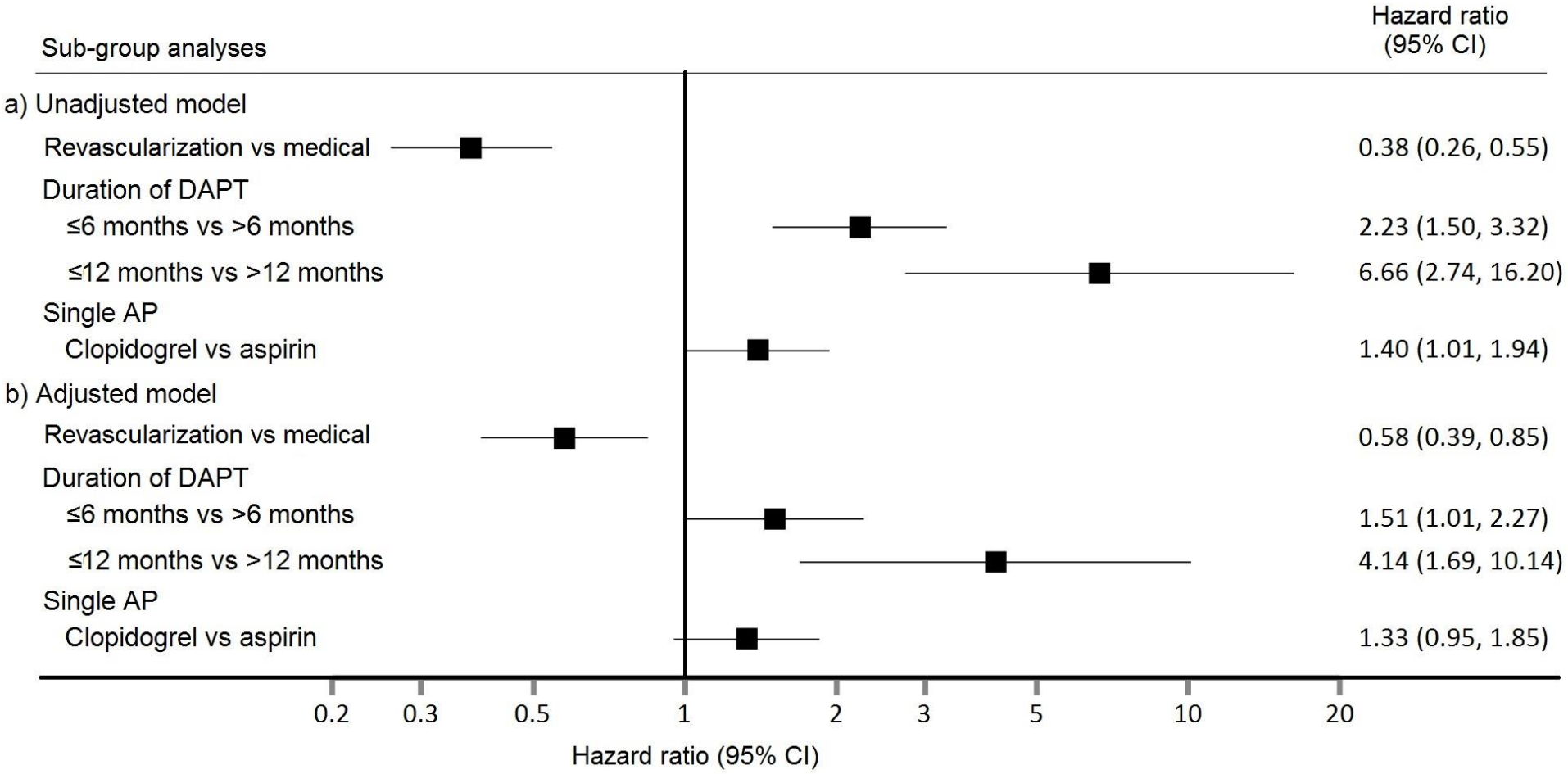


Figure 6-5: Forest plot showing outcomes for (a) unadjusted and (b) adjusted analysis.

All adjusted analyses were adjusted for age, medical or revascularization therapy and duration of DAPT. AP=antiplatelet, CI=confidence interval, DAPT=dual antiplatelet therapy.

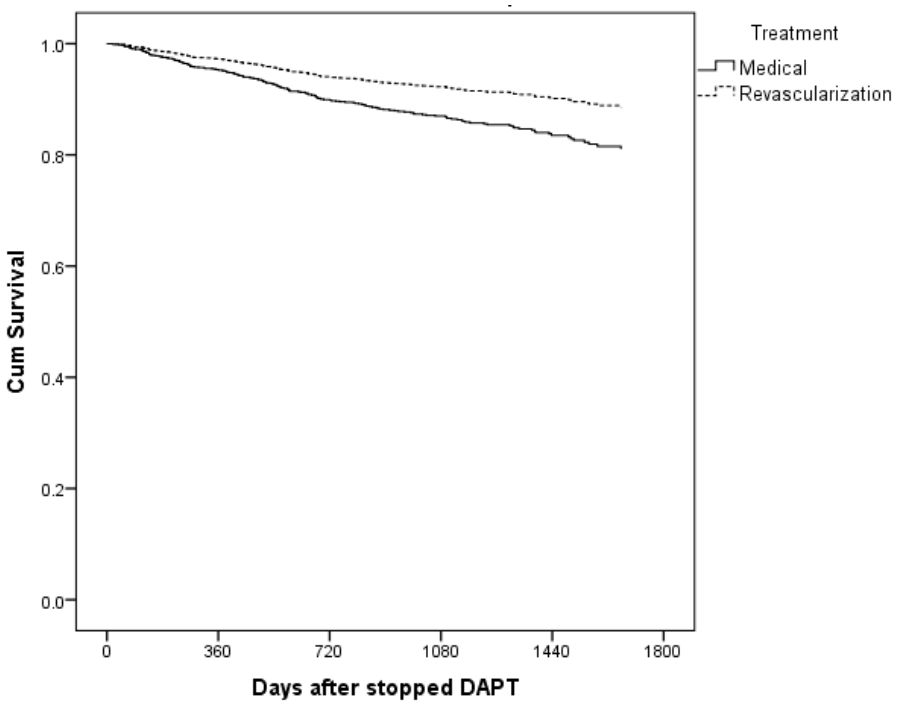
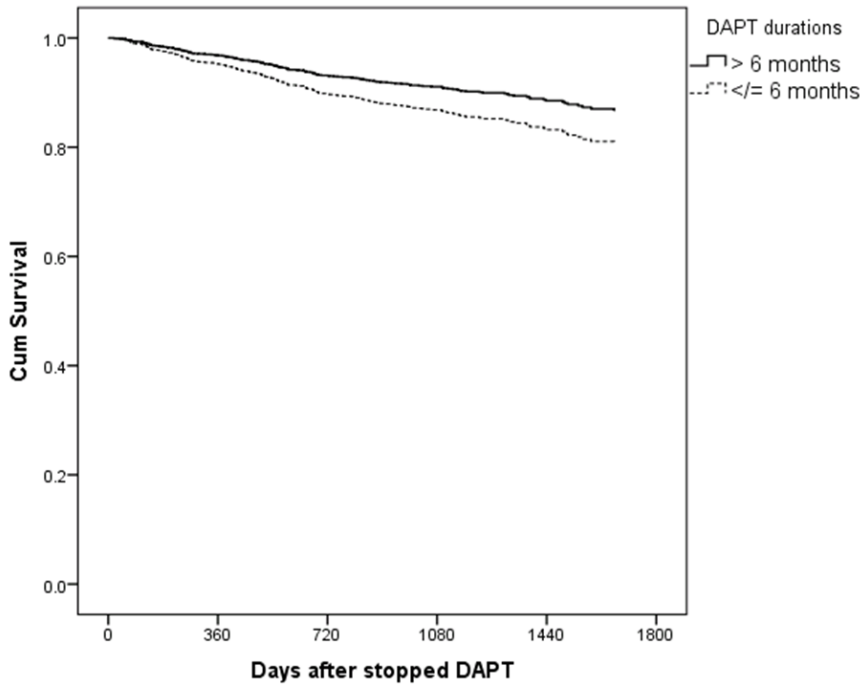


Figure 6-6: Survival curve showing the cumulative rates of the combined end point acute coronary syndrome or death or ischaemic stroke among those medically- and revascularization-treated patients after DAPT cessation

(a) ≤6 months vs >6 months



(b) ≤12 months vs >12 months

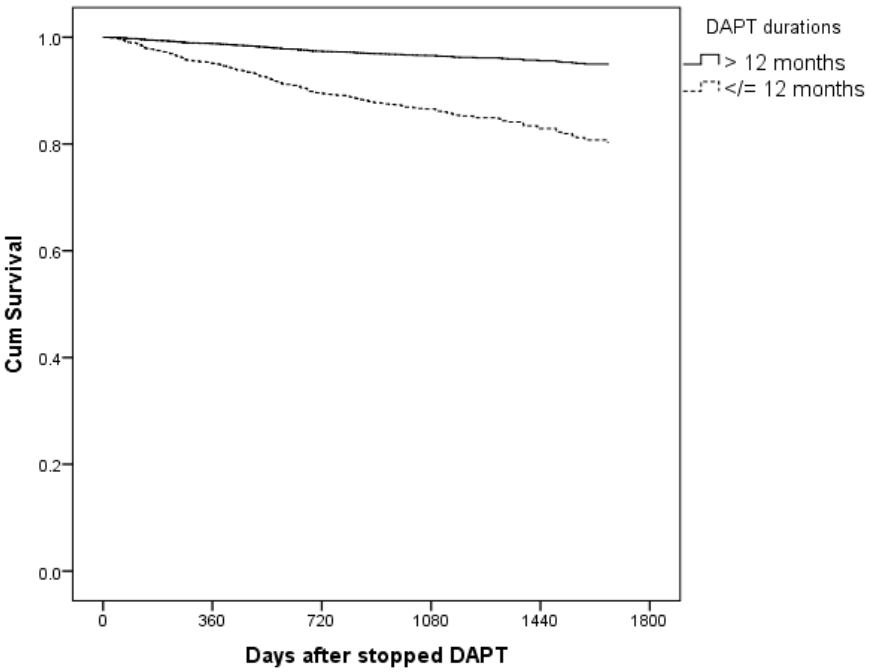


Figure 6-7: Survival curve showing the cumulative rates of cardiovascular events among those with DAPT duration (a) ≤ 6 months vs > 6 months; and (b) ≤12 months vs >12 months.

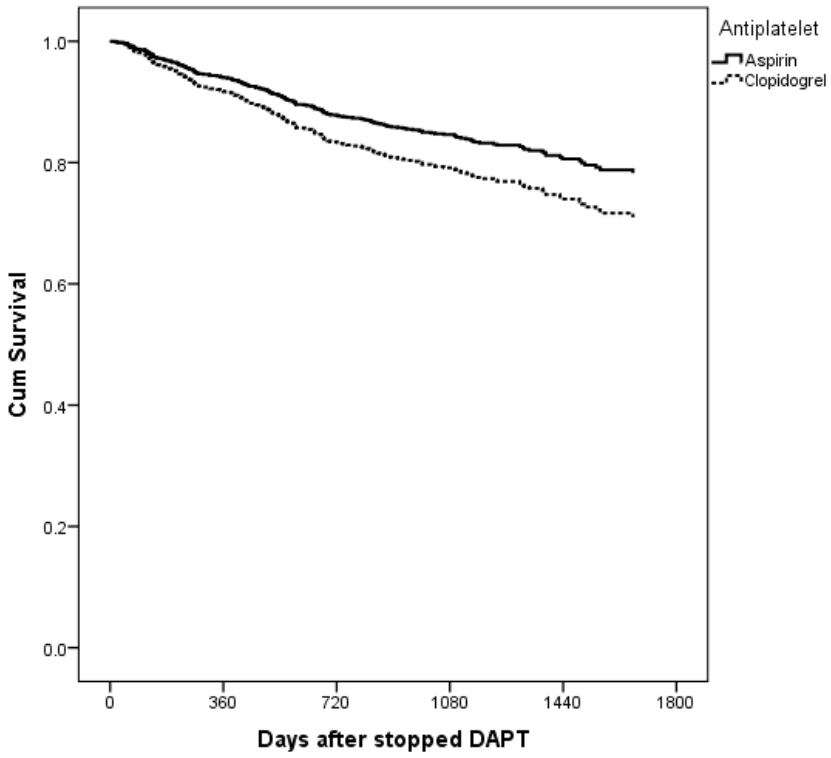


Figure 6-8: Survival curve showing the cumulative rates of the combined end point acute coronary syndrome or death or ischaemic stroke among those continued aspirin and those continued with clopidogrel after DAPT cessation.

6.4 Discussion

The objective of the present study was to determine the incidence and predictors of cardiovascular events after DAPT cessation in patients who have suffered ACS. The main findings of the current analysis are as follows; (1) incidence of cardiovascular events occurred in 188 (16.7%) patients after DAPT cessation; (2) the incidence rate was lower in 0-90-day interval than in the next 90-day interval after DAPT cessation, 0.15 and 0.23 per 1000 patient-days, respectively; (3) in the multivariate analysis, age, having medical or revascularization therapy and duration of DAPT were independent predictors of cardiovascular event after DAPT cessation; (4) duration of DAPT six months and less was associated a significantly higher risk for cardiovascular event; (5) continuation of monotherapy agent post-DAPT cessation with clopidogrel compared with aspirin was not associated with higher risk for cardiovascular event.

The incidence of cardiovascular events following DAPT cessation in the literature varies between studies (4-17%). The incidence in the present study are higher than

previous studies (Ho, et al., 2010; Stephenson, et al., 2011; Charlot, et al., 2012) but similar to the data from Ho, et al. (2008b). This may be due to differences in definitions used of cardiovascular event between studies that could lead to markedly different results (Kip, et al., 2008). In this study, cardiovascular event was defined according to ACC/AHA (Hicks, et al., 2015). On the other hand, the mean (SD) duration of DAPT after ACS in the present study was 177 (155) days, which was shorter than previous studies (Stephenson, et al., 2011; Charlot, et al., 2012). Previous studies have shown that thienopyridine therapy for more than six months was associated with lower rates of cardiovascular events (Rossini, et al., 2011; Varenhorst, et al., 2014).

The present study found the incidence rate of cardiovascular event in the first 90 days following discontinuation of DAPT was lower than 91-180-day interval. These findings are substantially different from those in recent studies from Ho, et al. (2008b); (Ho, et al., 2010); Stephenson, et al. (2011) and Charlot, et al. (2012) where they reported a higher incidence rate of death or MI in the 0-90-day interval after stopping clopidogrel compared with 91-180-day interval. This may be due to the smaller sample size of patients included in the present study. In addition, the prevalence of medically-treated patients in the present study was 67%, much higher than the findings from Ho, et al. (2008b) (50%) and Stephenson, et al. (2011) (25%). Evidenced-based data have shown that invasive strategy i.e. coronary angiography followed by revascularization performed in the setting of AMI reduces subsequent MI, severe angina and death compared with pharmacological therapy (Mehta, et al., 2005).

In terms of patients treated with or without revascularization, the present study found no increased incidence rate of cardiovascular event in the first 90-day period of DAPT discontinuation compared with later follow-up interval. There is a potential explanation for the lower number of cardiovascular events after stopping DAPT in the 0-90-day interval. One explanation is that there were maybe less patients in this study that may have stopped clopidogrel, prasugrel or ticagrelor early due to clinical deterioration or bleeding. They probably had stopped DAPT regimen under physician-guided or they had completed the recommended duration of DAPT. This is supported by findings from Mehran et al, 2013 in the PARIS (patterns of non-adherence to antiplatelet regimens in stented

patients) study where they found patients who had physician-guided discontinuation of DAPT were at lower risk of major cardiovascular event (Mehran, et al., 2013). In the present study, we also found the incidence rates in the cardiovascular event as time passed from the DAPT discontinuation were higher in patients treated medically compared to revascularization-treated patients. One possible reason is because revascularization-treated patients had longer DAPT duration compared to medically treated patients. Ho, et al. (2010) and Charlot, et al. (2012) have described that longer duration of DAPT was associated with lower risk of cardiovascular event following discontinuation of DAPT.

This study found that increasing age, shorter DAPT duration and lack of revascularization therapy for the ACS was associated with increased risk of cardiovascular events after DAPT cessation. The present study found that longer duration of DAPT was associated with a decreased risk of cardiovascular event in patients who have suffered ACS. In addition, shorter term DAPT (≤6 months) was associated with an increased risk of cardiovascular event compared to longer term (>6 months). Similarly, duration of DAPT ≤12 months was associated with an increased risk compared with >12 months. These findings are consistent with those from previous studies that demonstrated that usage of clopidogrel for more than six months was associated with lower rates of death, myocardial infarction and/or stent thrombosis (Rossini, et al., 2011; Varenhorst, et al., 2014). These data suggest that ACS patients may benefit from longer duration of DAPT. On the other hand, findings of PRODIGY trial found that there was no difference in death, MI or ischaemic stroke between DAPT duration of six months compared with 24 months (HR, 0.94; 95% CI, 0.69-1.27; p=0.67) (Costa, et al., 2015). Although longer treatment with DAPT might be beneficial, optimal DAPT duration for patients with ACS still in unclear because longer DAPT duration correlates with the risk of bleeding (Valgimigli, et al., 2012; Mauri, et al., 2014).

6.4.1 Study strengths

In this study, aspirin use was available through the Prescribing Information System database. Thus, we were able to confirm that all patients included in this study were taking aspirin plus either clopidogrel or prasugrel or ticagrelor. Compare to others, aspirin used was not available (Ho, et al., 2010; Stephenson, et al., 2011;

Sachdeva, et al., 2012). This information is important since there is a possibility that patients could not tolerate or allergic to aspirin and just prescribed with clopidogrel alone. Moreover, we could confirm whether patients took aspirin or another antiplatelet agent after discontinuing DAPT regimen.

As compared with others, the present study included patients in the West of Scotland as the data were collected and managed by NHSGGC Safe Haven. The current analysis is a study of all individuals in a community with an ACS identified through resources of the NHSGGC Safe Haven, regardless of sex and health care insurance. In previous studies, they only able to include predominantly male patients (Ho, et al., 2008b) or patients with insured and received care in large pre-paid health plan (Ho, et al., 2010; Stephenson, et al., 2011) and there was a possibility underinsured or non-insured were left out. Furthermore, the large number of patients, long period of follow-up, and use of Cox proportional hazards regression analysis in this study allowed us to develop more precise models to predict survival and cardiovascular events after stopping DAPT regimen.

6.4.2 Study limitations

There are limitations of the present study that must be considered. The analysis of this study was performed using a non-randomized registry database. The choice of antiplatelet used were not randomly assigned. Whereas for subgroup analysis, there are possibilities of patient selection bias and non-randomized groups. After DAPT discontinuation, the differences in time of follow-up for CV events is potential for biases. The use of single or dual antiplatelet therapy was based on pharmacy dispensing data. However, we could not measure medication compliance such as medication possession ratio (MPR), as this formula needs at least two prescriptions, and it is possible we will leave out any patients with only one prescription. Moreover, we had comprehensive pharmacy dispensing data in terms of what and when the antiplatelet was dispensed and number of pills supplied. Furthermore, pharmacy dispensing data are a validated measure of medication taking and is strongly correlated with a broad range of patient outcomes (Osterberg and Blaschke, 2005; Ho, et al., 2008a) and also, the use of prescriptions filled has been shown to reflect medication use by the patient with a high degree of accuracy (Lau, et al., 1997).

We also do not know the specific reasons for stopping the second antiplatelet therapy, but potential reasons are, they might have completed the DAPT course which is the expected reason for most of the patients; or the occurrence of side effects from antiplatelet therapy, such as bleeding. Additionally, the patients in the study sample were five years younger and less female than the original population. As a consequence, generalizability of findings in this study is limited. The present study included patients who have suffered ACS in the West of Scotland, thus, generalizability to other populations and practice setting is unknown.

6.5 Conclusion

The present study found that, in patients who have suffered ACS, incidence of cardiovascular events occurred in 188 (16.7%) patients after DAPT cessation and the incidence rate was lower in 0-90-day interval than in the next 90-day interval after DAPT cessation, 0.15 and 0.23 per 1000 patient-days, respectively. This study found that age was associated with increased risk of cardiovascular events, whereas, longer duration of DAPT and patients treated with revascularization were associated with decreased risk of cardiovascular events.

7 Stopping dual antiplatelet therapy following acute coronary syndrome; A metabolic analysis

7.1 Introduction

There have been reports of cardiovascular events after clopidogrel cessation among patients with ACS either received medical therapy or treated with PCI. In 2008, a study by Ho, et al. (2008b) using the cohort from Veterans Health Administration, found a clustering of cardiovascular events after clopidogrel cessation. In multivariate analysis, the study found the first 90-day interval after clopidogrel cessation was associated with significantly higher risk of cardiovascular events compared with 91-180 days interval (incidence rate ratio (IRR), 1.98; 95% confidence interval (CI), 1.46-2.69). Another study by Ho, et al. (2010) using cohort from an integrated health care delivery system in Colorado found similar observations. The risk of MI or death was greater (IRR, 2.74; 95% CI, 1.69 to 4.44) in day 0-90 compared with 91-360-day interval.

According to the current guidelines, antiplatelet drugs should be prescribed following acute myocardial infarction and ischaemic stroke (National Institute for Health and Clinical Excellence, 2008; National Institute for Health and Clinical Excellence, 2013). These include aspirin which reduces production of thromboxane A2 via inhibition of COX-1. Clopidogrel and ticagrelor bind to the $P2Y_{12}$-receptor on platelet cell membrane to prevent ADP secretion and thus, prevent platelet activation. Both aspirin and clopidogrel / ticagrelor are prescribed for varying durations after MI or ACS and at the end of this period the clopidogrel / ticagrelor is stopped. As described above, this may be associated with an increased risk of cardiovascular events.

Detailed metabolomic analysis in patients taking DAPT was performed before and after stopping DAPT. The metabolite changes between these two-time points will be compared. In this preliminary study, we hypothesized that metabolomics might help identify metabolites influencing outcomes and risk of recurrence following DAPT cessation.

7.2 Methods

7.2.1 Study design

The prospective study was conducted in the Western Infirmary and Queen Elizabeth University Hospital. Patients receiving DAPT with aspirin and clopidogrel or ticagrelor for a proposed fixed duration were recruited from the Cardiac Rehabilitation Centre and the wards of Western Infirmary (NHS Greater Glasgow & Clyde (NHSGGC)).

7.2.2 Main inclusion / exclusion criteria

Inclusion criteria for patients

1. Adult male or female
2. Age > 18 years
3. Diagnosis of ACS
4. Taking DAPT with aspirin and clopidogrel / ticagrelor since the diagnosis of ACS being made
5. Planned stop date for dual anti-platelet therapy

Exclusion criteria for patients

1. Current use of anticoagulant drugs
2. Active cancer
3. Recurrent urinary tract infection (UTI)*
4. Inability to provide informed consent

*For urinary metabolomics study, patients with recurrent UTI were excluded. This is because, these patients are at high risk of developing adverse event. Previous studies have shown that metabolites identified from patients with UTI, such as para-aminohippuric acid, scyllo-inositol and a few unidentified compounds has been suggested as main markers related to patients morbidity (Nevedomskaya, et al., 2012).

7.2.3 Ethical considerations

This study was carried on accordance with the World Medical Association Declaration of Helsinki (1964) and it's revisions (Tokyo (1975), Venice (1983), Hong Kong (1989), South Africa (1996), Edinburgh (2000), Washington (2002), Tokyo (2004), Seoul (2008) and Fortaleza (2013)). Patients were only allowed to enter the study once they have provided written informed consent. This study was approved by the North West – Greater Manchester East Research Ethics Committee (**Appendix 9** & **Appendix 10**). Access to the hospital for research was obtained from the Research and Development Office, Western Infirmary (**Appendix 11**).

7.2.4 Identification of participants and consent

Participants were identified through clinical teams in the Cardiac Rehabilitation Centre and the wards of the Western Infirmary. Study advertisements were placed in these settings and interested patients were asked to contact the study team (**Appendix 12**). Letter of invitations were sent from the clinical team to patients who met our inclusion and exclusion criteria (**Appendix 13** & **Appendix 14**). Interested patients were asked to contact us. We then discussed the study by telephone, confirmed the predicted DAPT stop date and sent the participant information sheets (**Appendix 15**).

7.2.5 Visit schedule

Participants attended the Acute Stroke Unit, Western Infirmary or Glasgow Research Facility, Queen Elizabeth University Hospital for a first visit in the month prior to the planned DAPT stop date. All participants provided written informed consent (**Appendix 16**) prior to study inclusion. At this visit, baseline demographic data, current medication, use of non-steroidal anti-inflammatory medications and medical history were recorded in the case report form (CRF) (**Appendix 17**). Medication compliance was recorded and assessed by self-reported Morisky scale questionnaire (**Appendix 18**). Blood and urine sampling were performed during this visit (Figure 7-1).

Participants then attended the Acute Stroke Unit, Western Infirmary or Glasgow Research Facility, Queen Elizabeth University Hospital for a further blood and urine sample in the month after stopping DAPT (at least 7 days after stopping). Current medications were recorded in the CRF. A 90-day followed up were done by accessed to participant clinical records.

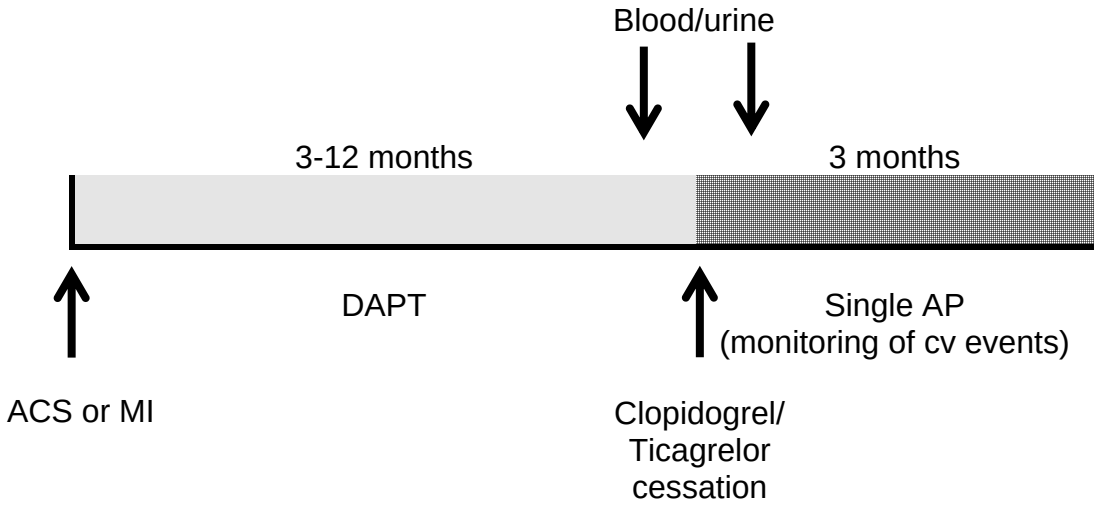


Figure 7-1: Study design and protocol of laboratory and monitoring cardiovascular events.

ACS=acute coronary syndrome, AP=antiplatelet, cv=cardiovascular, DAPT=dual antiplatelet therapy, MI=myocardial infarction.

7.2.6 Blood sampling and storage

I personally performed venepuncture in approximately 90% of the participants (the remaining 10% were performed by experienced research nurses from Acute Stroke Unit). Blood samples were obtained from the antecubital fossa. In all cases blood was taken using a tourniquet with a 21-gauge needle blood collection set. The blood was drawn into two 10 ml clot activator tubes.

In metabolomics studies, serum and plasma samples are commonly used. In this study, serum sample was chosen. Once the blood exposed to clot activator in the tube, platelets become activated. In the serum, various of metabolites, lipids and proteases were released from the activated platelets during the coagulation process (Yin, et al., 2015). On the other hand, exposure of plasma for long hours

to room temperature may resulted significant changes of the analysed metabolites (Yang, et al., 2013).

The blood was allowed to clot at room temperature for 15 minutes. Then, blood samples were centrifuged for 15 minutes. Serum samples were separated and stored at -80˚C immediately. Samples were stored in a locked university freezer in the Western Infirmary and were transported at the end of the study to the Glasgow Polyomics facility at the University of Glasgow.

7.2.7 Assessment of medication compliance

Self-reported adherence was determined using Morisky Medication Adherence Scale-4 (MMAS-4) at Visit 1. The MMAS-4 is a validated, 4-item self-reported adherence measure and a high adherence will be defined as a zero point (**Appendix 18**) (Morisky, et al., 1986). This scale has been shown to be predictive of adherence to cardiovascular medication and blood pressure control (Morisky, et al., 1986; Shalansky, et al., 2004).

7.2.8 Assessment of cardiovascular event / follow up

Patients were followed-up for cardiovascular event through a telephone call at day 90 or via access to their clinical records if contact could not be made. They were asked if there was any admission within the last 90 days and then we accessed patient medical records. If they developed event(s), their types of events were recorded in their CRF.

7.2.9 Definition of cardiovascular event

Cardiovascular event was defined as re-admission to hospital for a cardiovascular event, which includes ACS, stroke, TIA, heart failure, stent thrombosis or death due to a cardiac cause or stroke. The cardiovascular event outcomes were based on a primary discharge diagnosis or death using the ICD-10 codes in **Appendix 5**.

7.2.10 Metabolite measurements by LCMS

Once transferred to Glasgow Polyomic facility, serum samples were mixed as Chloroform : Methanol : sample in a 1:3:1 ratio. This was then mixed vigorously on a cooled (4°C) shaker for 5 minutes and then centrifuged for 3 minutes at 13,000g at 4°C. Supernatant was removed and stored at -80°C until analysis by Liquid Chromatography Mass Spectrometry (LC-MS). Samples were then analysed using ultra-high resolution mass spectrometry.

7.2.11 Quality control (QC)

In this untargeted metabolomics approach by LC-MS, QCs were applied to assess and ensure the process of analytical is done appropriately. The pooled QC samples were used to assess the running of the instrument for reproducibility. The pooled samples were run throughout the sample analysis, every 5th sample.

7.2.12 Liquid chromatography-mass spectrometry

The sample platform chosen for this project was liquid chromatography coupled with mass spectrometry. Metabolites were separated using a ZIC-pHILIC column (150mm x 4.6mm, 5µm column, Merck Sequant) on a Dionex Ultimate 3000 RSLC system (Thermo Fisher Scientific, Hemel Hempstead, UK). Analysis was performed using 10 µl injection volume and a flow rate of 300 µl/min. For mass spectrometry analysis, a Thermo Orbitrap Exactive (Thermo Fisher scientific) was operated in both positive and negative detection ion modes. The work was done by researchers at the Glasgow Polyomics Facility.

7.2.13 Metabolomic data analysis

Samples for Metabolomic Analysis

Limits of quantitation (LOQ) parameter, the smallest concentration of analyte, was set at 1000 intensity. Samples detected below of this level were excluded from data analysis.

Data Processing

Data were analysed using an in-house suite of software built using XCMS (Smith, et al., 2006), MzMatch (Jankevics, et al., 2012) and IDEOM version 18 software. The software consists of state-of-the-art peak-picking, alignment and filtering algorithms that effectively mine raw metabolomics data to produce meaningful information. A full suite of statistical tools is also built in to the software.

7.2.14 Statistical analysis

Descriptive statistics were recorded. For categorical variables such as demographics and medical history data, they were summarised using frequencies and proportions. Whereas for continuous variables, they were summarised using mean [standard deviation (SD)] or median [interquartile range (IQR)].

This is an exploratory study so no sample size calculation was performed. There are no data available on the metabolic signature of clopidogrel and/or ticagrelor while taking these two antiplatelet agents and after discontinuation. The baseline time refers to before clopidogrel or ticagrelor cessation (pre-). The differences of metabolite changes before and after DAPT cessation was determined using paired t-test with a p-value of <0.05 was considered to represent statistical significance.

7.3 Results

7.3.1 Participant characteristics

A total of 29 patients were screened in the study. One patient withdrew prematurely due to inconvenience associated with multiple hospital visits required for venesection, 11 patients were excluded because they did not know the planned stop date for clopidogrel/ticagrelor and there were 7 patients where the durations of clopidogrel/ticagrelor were longer than the study duration. Study data are presented on 10 patients.

Baseline characteristics and demographics of the study population are provided in Table 7-1. The participants were predominantly male (60%) with a mean age (SD)

of 64.6 (7.2) years. Cardiac risk factors included hyperlipidaemia (30%), hypertension (20%) and active smoking (20%). All participants were on aspirin 75mg daily, statin and beta-blocker therapy. Nine (90%) patients were on ticagrelor 90mg twice daily, one (10%) patient was on clopidogrel 75mg daily, 7 (70%) were on angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) and 4 (40%) were on proton pump inhibitors. The mean total duration (SD) of DAPT therapy following ACS was 174 (28.4) days.

Table 7-1: Baseline characteristics

Characteristics	All patients n=10	Metabolomics analysis n=7
Age, years		
Mean (SD)	64.6 (7.152)	66.0 (7.188)
Median (IQR)	65.5 (59-70)	68 (60-72)
Sex		
Female	4 (40)	3 (42.9)
Male	6 (60)	4 (57.1)
Current smoker	2 (20)	1 (14.3)
Medical history		
Hypertension	2 (20)	2 (28.6)
Diabetes	0 (0)	0 (0)
Hyperlipidaemia	3 (30)	2 (28.6)
Stroke/TIA	1 (10)	1 (14.3)
IHD	1 (10)	0 (0)
LV systolic function (LVEF)		
Good (LVEF >50%)	9 (90)	6 (85.7)
Moderate (LVEF 30-50%)	1 (10)	1 (14.3)
Poor (LVEF <30%)	0 (0)	0 (0)
Indication for DAPT		
ST-elevation MI	5 (50)	5 (71.4)
Non-ST-elevation MI	3 (30)	2 (28.6)
Unstable angina	1 (10)	0 (0)
ACS	1 (10)	0 (0)
DAPT duration, days		
Mean (SD)	173.5 (28.383)	167.4 (32.501)
Median (IQR)	182.5 (172-188)	180 (171-184)
Antiplatelet therapy		
Aspirin	10 (100)	7 (100)
Clopidogrel	1 (10)	0 (0)
Ticagrelor	9 (90)	7 (100)
Other medication		
Statin	10 (100)	7 (100)
Beta-blockers	10 (100)	7 (100)
ACE-inhibitors/ARB	7 (70)	5 (71.4)
Diuretics	1 (10)	1 (14.3)
Proton pump inhibitors	4 (40)	3 (42.9)

All values are reported as no. (%) unless otherwise noted. ACE=angiotensin-converting enzyme, ACS=acute coronary syndrome, ARB=angiotensin receptor blocker, DAPT=dual antiplatelet therapy, IHD=ischaemic heart disease, IQR=inter-quartile range, LV=left ventricular, LVEF=left ventricular ejection fraction, MI=myocardial infarction, PCI=percutaneous coronary intervention, SD=standard deviation, TIA=transient ischaemic attack. *All patients. †After exclusion.

7.3.2 Clinical events

During the study follow-up, no patients developed subsequent cardiovascular event as defined in section 7.2.9. However, there was one patient admitted to the hospital, for one night, 12 days after stopping clopidogrel with a complaint of left leg pain and swelling.

7.3.3 Changes in metabolite levels after discontinuation of DAPT

For metabolomics analysis, three patients were removed from the analysis i.e. one patient treated with clopidogrel and two patients with poor signal (LOQ < 1000). Thus, metabolomics analysis data are presented for 7 patients. Baseline characteristics and demographics for 7 patients as shown in Table 7-1.

In metabolomics analysis, 311 putative metabolites were identified (**Appendix 19**). Figure 7-2 shows the distribution of metabolites identified before and after DAPT cessation according to their grouped pathways. The majority of metabolites identified were from lipid pathway (39%), which most of them came from glycerophospholipid and fatty acyls pathways. Amino acid pathway accounted for about 22% and followed by carbohydrate metabolism, at 8.4%. There were 23% metabolites from unknown pathways. The remaining 8% metabolites were from nucleotide, cofactors and vitamins, peptide, energy and biosynthesis of secondary metabolites pathway.

Data analysed using IDEOM v18 found 16 putative metabolites significantly altered by DAPT discontinuation in the seven included patients (Table 7-2). Of these, 7 metabolites were from lipid pathway, 1 from carbohydrate metabolism, 1 from nucleotide metabolism. Lipid pathway was significantly affected by DAPT cessation. Most of the compounds identified were downregulated especially compounds in lipid pathway, which downregulated some up to 3-fold. Whereas for adenosine, metabolite identified from nucleotide metabolism, was upregulated 2.6-fold.

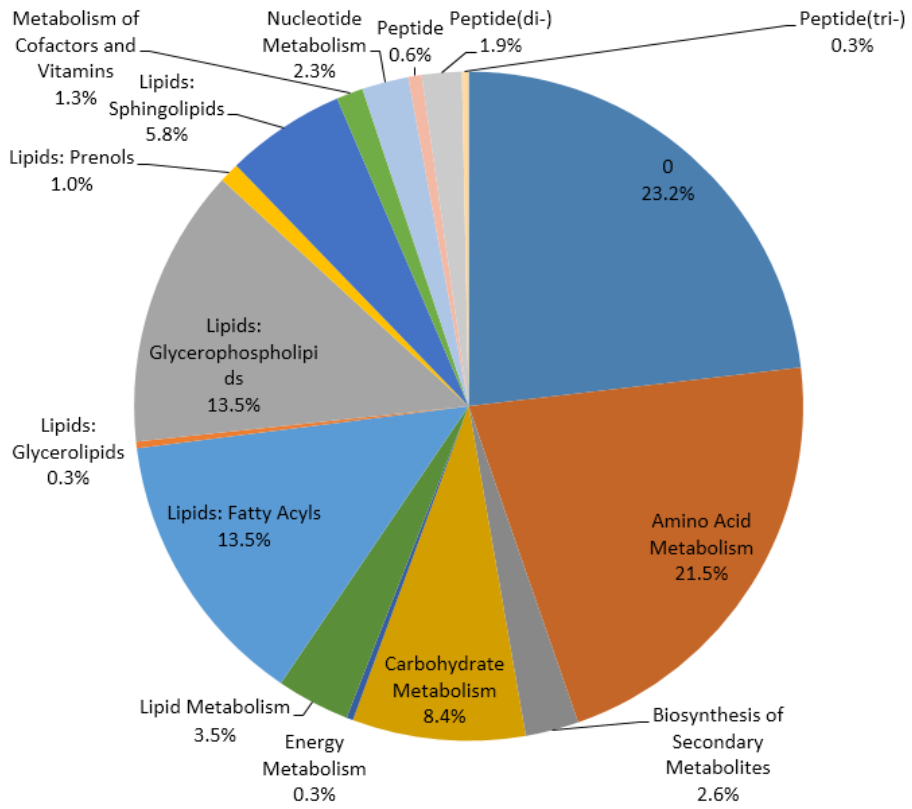


Figure 7-2: Pie chart of putative metabolites identified according to their group of pathway following DAPT cessation in ACS patients.

There were 7 compounds identified from lipid pathway which majority came from fatty acyls metabolism, and the remaining from lipid metabolism and sphingolipid metabolism. The metabolites were 4-8dimethylnonanoylcarnitine, 10-undecenoic acid, 2-methyl-2-dodecenoic acid, 9,12-hexadecadienoic acid, 9Z,12Z,15Z-octadecatrienoic acid, 11Z-eicosenoic acid and sphingomyelin. Figure 7.3 shows the graphs of seven individual metabolites identified before and after DAPT cessation from lipid pathways with mean peak intensities and error bars. As we can see, the mean intensity for octadecatrienoic acid compound was the highest compared to others even before and after DAPT cessation.

Table 7-2: Significant metabolites changes detected following DAPT cessation.

Formula	Putative metabolite	KEGG ID	Map/Pathway	Post-DAPT cessation	p-value*†
ClHO4	perchlorate	-	0	0.82	0.010
C21H41NO4	Tetradecanoylcarnitine	-	0	0.62	0.048
C19H37NO4	1,2-dioctanoyl-1-amino-2,3-propanediol	-	0	0.59	0.050
C12H18O2	4-Hexyloxyphenol	C14305	0	0.59	0.024
C21H37NO4	3, 5-Tetradecadiencarnitine	-	0	0.50	0.037
C29H48O2	(24R,24'R)-Fucosterol epoxide	C03910	0	0.40	0.035
C10H12O3	Coniferyl alcohol	C00590	Biosynthesis of Secondary Metabolites	0.48	0.046
C6H14O6	D-Sorbitol	C00794	Carbohydrate Metabolism	0.26	0.032
C18H35NO4	4-8dimethylnonanoylcarnitine	-	Lipid Metabolism	0.63	0.002
C11H20O2	[FA (11:1)] 10-undecenoic acid	C13910	Lipids: Fatty Acyls	0.67	0.041
C13H24O2	[FA methyl(12:1)] 2-methyl-2-dodecenoic acid	-	Lipids: Fatty Acyls	0.67	0.039
C16H28O2	[FA (16:2)] 9,12-hexadecadienoic acid	-	Lipids: Fatty Acyls	0.52	0.041
C18H30O2	[FA (18:3)] 9Z,12Z,15Z-octadecatrienoic acid (alpha-Linolenic acid)	C13910	Lipids: Fatty Acyls	0.47	0.037
C20H38O2	[FA (20:0)] 11Z-eicosenoic acid	C13910	Lipids: Fatty Acyls	0.44	0.034
C41H81N2O6P	SM(d18:1/18:1(9Z)) (Sphingomyelin)	C13910	Lipids: Sphingolipids	0.78	0.044
C10H13N5O4	Adenosine	C13910	Nucleotide Metabolism	2.60	0.028

Metabolites identified with confidence 10 (matching authentic standard) are highlighted in grey. Data were generated on IDEOM v18. *Paired t-test was performed to calculate p-value. †uncorrected p-value.

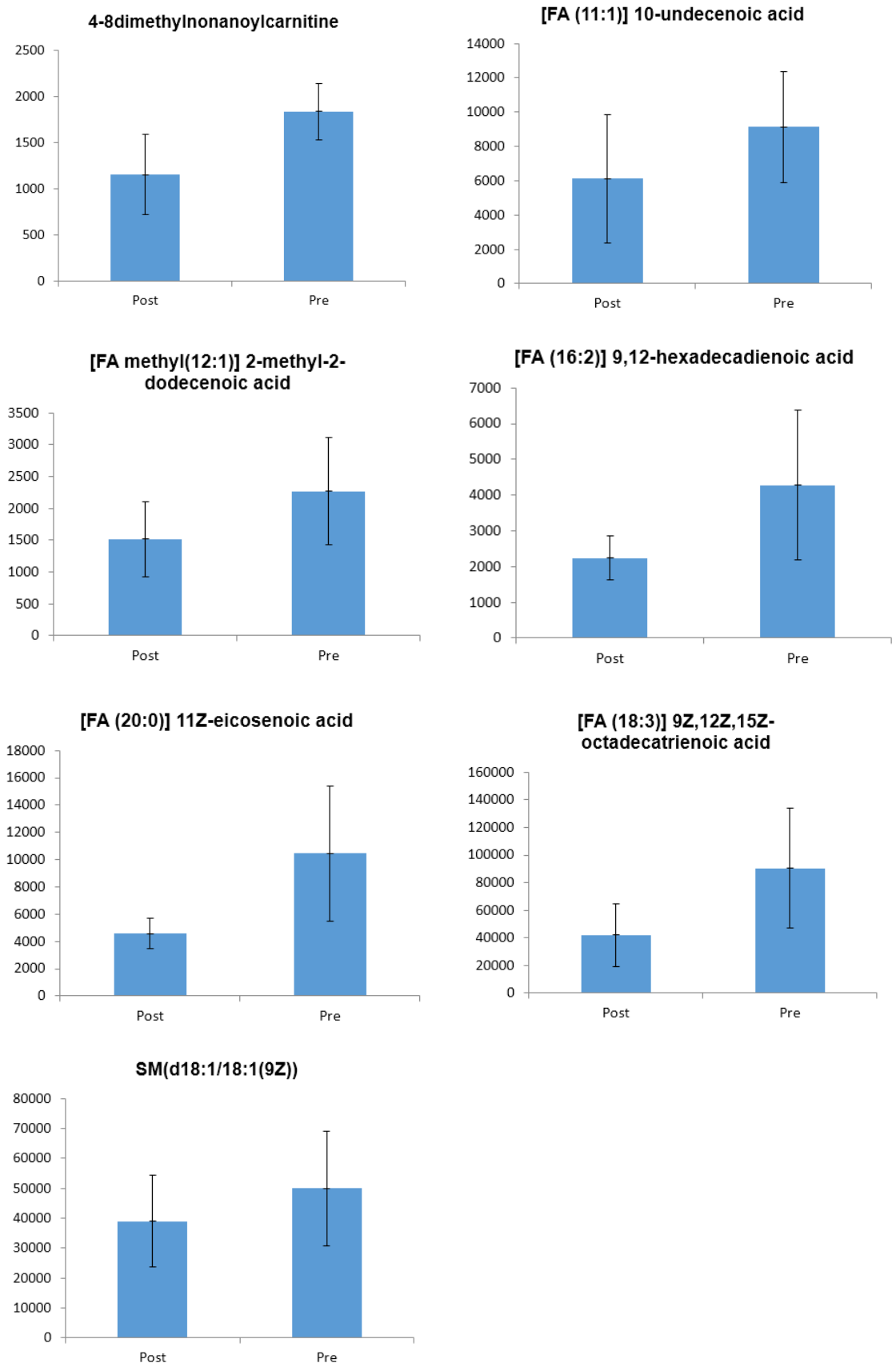


Figure 7-3: Fatty acid metabolism differences following DAPT cessation in patients with ACS. Graphs showing the mean intensity of compounds of before (pre) and after (post) DAPT cessation, with error bars.

Table 7-3 shows 7 putative metabolites identified from nucleotide metabolism. Five metabolites were downregulated and two metabolites were upregulated. Adenosine was found significantly altered following DAPT cessation (p=0.028) and inosine was a borderline significant trend (p=0.082). The remaining metabolites in this pathway were not significantly associated with ticagrelor cessation. Mean peak intensities and error bars for adenosine compound is presented in Figure 7.4. The mean intensities for adenosine was increased from 2042 to 5306 following ticagrelor cessation.

Table 7-3: Nucleotide metabolism differences after DAPT cessation.

Formula	Putative metabolite	Post-DAPT cessation	p-value*†
C10H13N5O4	Adenosine	2.60	0.028
C5H4N4O2	Xanthine	1.05	0.868
H2O4S	Sulfate	0.88	0.276
C5H4N4O	Hypoxanthine	0.88	0.793
C9H12N2O6	Uridine	0.81	0.538
C10H12N4O5	Inosine	0.78	0.082
C5H4N4O3	Urate	0.78	0.172

Metabolites identified with confidence 10 (matching authentic standard) are highlighted in grey. Significant metabolites changes are highlighted in blue. Data were generated on IDEOM v18. *Paired t-test was performed to calculate p-value. †uncorrected p-value.

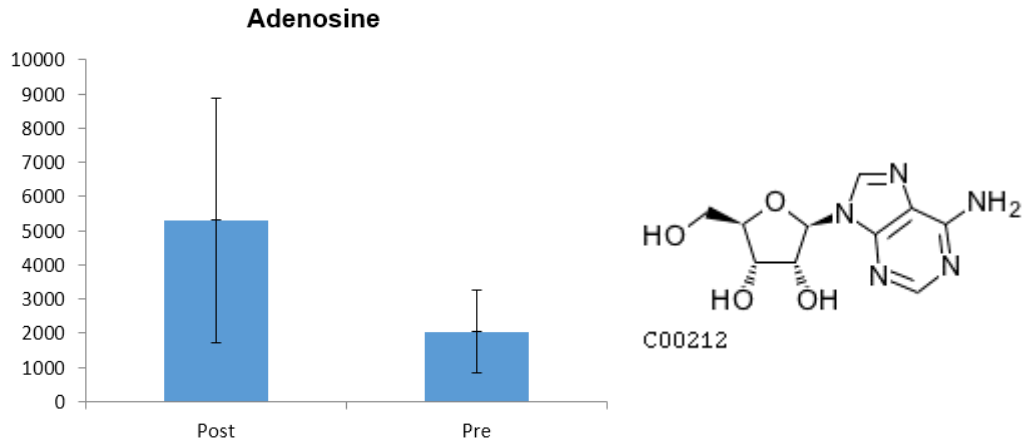


Figure 7-4: Adenosine compound differences following DAPT cessation in patients with ACS. Graph showing the mean intensity of compound of before (pre) and after (post) DAPT cessation, with error bars.

Only one putative metabolite was significant from carbohydrate metabolism i.e. D-sorbitol (Table 7.4). The mean intensities for D-sorbitol was decreased from 37482 to 9642 following ticagrelor cessation (Figure 7-5).

Table 7-4: Changes detected in carbohydrate metabolism after DAPT cessation.

Formula	Putative metabolite	Post-DAPT cessation	p-value*†
C4H8O2	Butanoic acid	0.98	0.831
C4H8O2	(R)-Acetoin	0.97	0.421
C6H12O7	D-Gluconic acid	0.94	0.696
C6H12O6	myo-Inositol	0.93	0.610
C6H10O5	2-Dehydro-3-deoxy-L-rhamnonate	0.90	0.547
C5H10O5	D-Xylulose	0.88	0.532
C4H8O4	D-Erythrose	0.88	0.461
C3H4O3	3-Oxopropanoate	0.87	0.488
C6H6O6	cis-Aconitate	0.87	0.703
C5H8O4	2-Acetolactate	0.87	0.512
C5H10O4	Deoxyribose	0.85	0.710
C5H10O5	D-Ribose	0.84	0.583
C5H10O6	L-Arabinonate	0.84	0.425
C6H13NO5	D-Galactosamine	0.84	0.391
C6H12O5	L-Rhamnulose	0.84	0.318
C5H6O5	2-Oxoglutarate	0.79	0.510
C6H8O7	Citrate	0.75	0.374
C4H8O3	4-Hydroxybutanoic acid	0.75	0.330
C4H6O5	(S)-Malate	0.74	0.570
C4H8O5	[FA trihydroxy(4:0)] 2,3,4-trihydroxy-butanoic acid	0.74	0.209
C3H8O3	Glycerol	0.71	0.186
C5H6O6	4-Hydroxy-2-oxoglutarate	0.68	0.399
C3H4O3	Pyruvate	0.66	0.484
C3H6O3	(R)-Lactate	0.51	0.420
C6H8O6	Ascorbate	0.47	0.430
C6H14O6	D-Sorbitol	0.26	0.032

Metabolites identified with confidence 10 (matching authentic standard) are highlighted in grey. Significant metabolites changes are highlighted in blue. Data were generated on IDEOM v18. *Paired t-test was performed to calculate p-value. †uncorrected p-value.

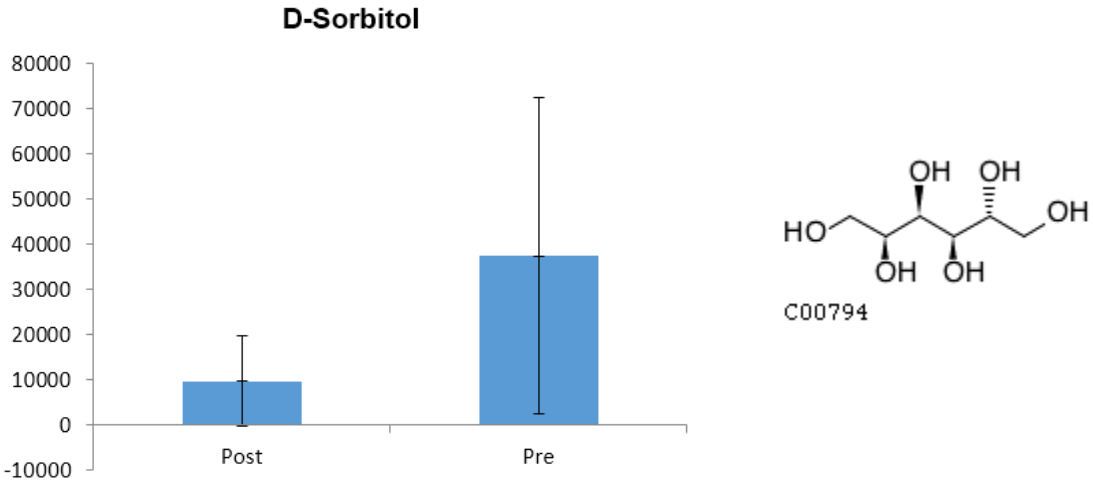


Figure 7-5: D-sorbitol compound differences following DAPT cessation in patients with ACS. Graph showing the mean intensity of D-sorbitol before (pre) and after (post) DAPT cessation, with error bars.

7.4 Discussion

Ten ACS patients were recruited in this preliminary study. All patients were treated with aspirin 75mg daily and combination with thienopyridine; nine patients were on ticagrelor and only one patient was on clopidogrel. One of the objectives of the study was to see metabolite changes after clopidogrel cessation, as clopidogrel cessation are known to cause increased risk of cardiovascular event, especially within the first 90 days after discontinuation. However, due to changes in clinical practice with the approval of ticagrelor for the treatment of post-PCI and ACS in the United Kingdom in October 2011 (National Institute for Health and Clinical Excellence, 2011), there were more patients prescribed with ticagrelor than clopidogrel in this study. Thus, for metabolomics analysis purposes, one patient treated with clopidogrel and two patients with poor signals were excluded from the analysis. Although at the moment, there is no evidence of increased risk of cardiovascular event following discontinuation of ticagrelor, in this section, discussion will be focusing on metabolite changes related to ticagrelor.

In the present pilot study, the level of adenosine was significantly increased and inosine level was decreased with almost approached significance, following ticagrelor cessation. These findings are not completely understood. Logically, adenosine level should decrease following ticagrelor cessation as others (van Giezen, et al., 2012; Armstrong, et al., 2014; Bonello, et al., 2014) have reported that ticagrelor increases adenosine concentration in ACS patients via inhibiting cellular uptake of adenosine (refer Figure 7-6). Although further study is needed, there are possible mechanisms that maybe responsible for these findings. First, adenosine levels in the extracellular space mainly derive from phosphor-hydrolysis of precursor nucleotides such as adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) (Eltzschig, 2009) (Figure 7-6). Thus, it is speculated that there is on-going platelet activation secondary to exposure with the damaged endothelium tissue. Studies by Schultheiss, et al. (1994) and Ault, et al. (1999) supported that platelet remains activated within one month following ACS, in patients with stable coronary artery diseases (CAD) (Furman, et al., 1998) and remains activated up to six months following STEMI (Scalone, et al., 2011). Under conditions of endothelium injury, adenosine is released at sites of tissue injury through degradation of released ATP and ADP

(Park and Gupta, 2013). Second, extracellular adenosine half-life is about few seconds (Moser, et al., 1989) as it is quickly taken up by cells and break down to inosine (Park and Gupta, 2013), thus, there is a possibility of poor uptake of adenosine by cells such as platelets and erythrocytes. Finally, another possible explanation is due to a low level of enzyme adenosine deaminase (ADA) that converts adenosine to inosine or adenosine kinase (ADK) that converts adenosine to AMP, could contribute to increase level of adenosine. Thus, it is speculated that with increased intracellular adenosine level, it will be transported via sodium-independent equilibrative nucleotide transporter (ENT1) to extracellular and thus increased extracellular adenosine. High level of extracellular adenosine might not be harmful to patients, but enhance the biological effects of adenosine.

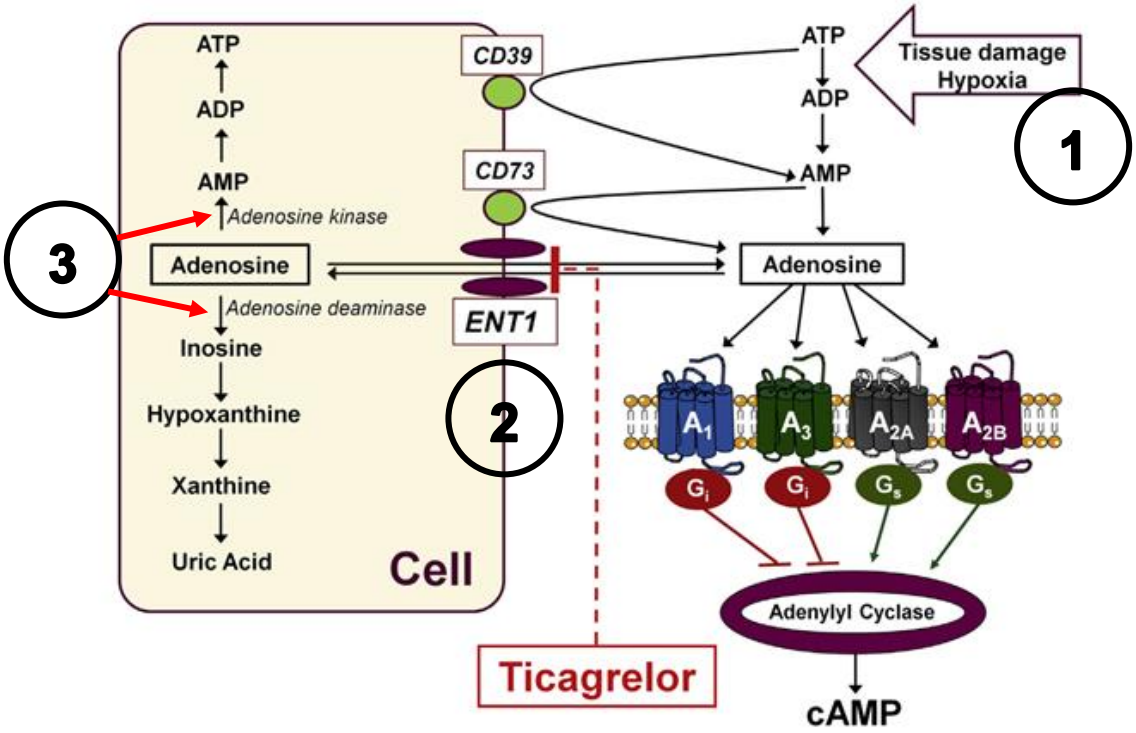


Figure 7-6: Formation of adenosine and its intracellular uptake and metabolism, which are reduced by ticagrelor through inhibition of ENT1.

Possibilities of contribution of increase level of adenosine following ticagrelor cessation, 1) on-going tissue damage, 2) poor uptake of adenosine, 3) low level of adenosine kinase or adenosine deaminase. Adapted from Cattaneo, et al. (2014) (Permissions obtained from Elsevier, License Number 3827770177972)

In this observational pilot study, several lipid compounds levels were decreased after ticagrelor cessation. One putatively identified compound was sphingomyelin. Sphingomyelin level was decreased significantly from the baseline. Although this result is not completely understood, there is one possible explanation for this, i.e. it is because all ACS patients in this study were treated with a cholesterol-lowering agent i.e. simvastatin or atorvastatin. Sphingomyelin is known to associate with cardiovascular disease risk (Stegemann, et al., 2014). Jiang, et al. (2000) found that plasma sphingomyelin was significantly higher in patients with CAD and few years later, Schlitt, et al. (2006) found that sphingomyelin was associated with myocardial infarction and cardiovascular death in ACS patients. Another study by Chen, et al. (2011) found sphingomyelin levels were higher among patients with CHD and in those with left ventricular (LV) systolic dysfunction (LVEF<50%) than in patients without CHD or LV dysfunction. It is speculated that statin therapy decreased level of sphingomyelin. Sphingosine-1-phosphate (S1P), a major metabolite produced following sphingomyelin degradation, activates endothelial NO synthase (eNOS) in vascular endothelial cells by binding to G-protein-coupled S1P1 receptors (Igarashi, et al., 2001; Igarashi and Michel, 2001; Igarashi, et al., 2003). Igarashi, et al. (2007) found that statins increased eNOS activity in response to stimulation with S1P and upregulated S1P1 receptor expression, thus, this may lead to metabolism of sphingomyelin and decrease sphingomyelin's level following statin therapy.

Although, only 16 putative metabolites significantly altered following ticagrelor cessation, it is important to note that there are another 19 putative metabolites that almost reach nominal significance (p-value between 0.051 to 0.10) in this study still may be scientifically important and bear further investigation. Future works on targeted analyses may be necessary to support the findings from this study. Moreover, the findings in this study do not only provide metabolite changes detected following ticagrelor discontinuation, but also provide metabolic signatures of ticagrelor response. Metabolomics, the study of metabolism, can help us identify pathways involved in drug clinical efficacy as well as risk of cardiovascular events associated with antiplatelet therapy (Lewis, et al., 2013; Yerges-Armstrong, et al., 2013; Ellero-Simatos, et al., 2014).

7.4.1 Strengths and limitations

This preliminary study has several strengths. First, this study was performed in ACS patients, and thus it able to reflect the effect of ticagrelor cessation in patients with ischaemia and coronary artery disease. Second, untargeted metabolomics approach by LCMS is able to generate rich metabolomics data, for example in this study, 311 putative metabolites were detected.

There are limitations in this present work. Firstly, it describes a clinical observation in a small number of patients. There are challenges in recruiting research participants. Many potential participants did come forward after received invitation letters but many were ineligible as they were not sure the date of stopping DAPT or were unable to attend as they need to travel long distances for study visits. Although with a small sample size, this study collected samples from multiple time-points for a single subject, which able to reduce the variability level (B.Dunn, et al., 2013). Secondly, sampling bias is likely in this study. To increase the number of recruitment and to make the study more attractive, the study allowed the two visits at any time of the day. There are many factors influence metabolite profiles such as dietary intake and what time they eat (Park, et al., 2009). Nevertheless, the study collected blood samples under a similar condition where all patients were allowed to eat and drink as normal.

7.5 Summary

This is a preliminary report on multiple metabolites changes following DAPT cessation in a small sample of patients with ACS. Findings suggest the possibility of relevant changes in platelet related metabolism post-DAPT cessation. A long-term goal is to associate metabolites changes with cardiovascular event post-DAPT cessation.

8 Conclusion

Antiplatelet therapy is the cornerstone of secondary prevention following an acute coronary syndrome (ACS) or ischaemic stroke, and antiplatelet agents have been shown to improve clinical outcome in these conditions. However, despite the use of antiplatelet therapy in these populations, cardiovascular events still occurred. Several studies have been carried out to assess certain clinical aspects when using antiplatelet in patients who have suffered ACS or ischaemic stroke.

Antiplatelet therapy is normally prescribed as a secondary prevention medication in order to prevent recurrent event, however, there are patients who experience a stroke whilst taking antiplatelet. So in chapter 3, the Virtual International Stroke Trials Archive (VISTA) data were used to test whether those who change to a new antiplatelet regimen following acute stroke have better clinical outcomes. The study found that patients who changed their pre-existing antiplatelet therapy was not associated with a lower risk of recurrence stroke than continuing on the same antiplatelet regimen. Patients who changed to a new antiplatelet regimen was associated with more favourable functional outcome at day 90, although this observation might be due to confounding at the baseline. The results may have relevance to the clinical practice, yet, the selection of an antiplatelet agent should be individualized based on patient characteristics.

Guidelines suggest initiation of antiplatelet therapy within 24 to 48 hours following acute stroke or it must be avoided for 24 hours following thrombolytic therapy. In chapter 4, the study examines whether early initiation (initiated on the same or following day after stroke) of antiplatelet therapy associated with better clinical outcomes. The study observed, in patients who have suffered ischaemic stroke, early initiation of antiplatelet therapy was not associated with better outcomes than later initiation. Furthermore, early initiation was associated with an increased risk of bleedings. Although, the results might be applicable to clinical practice, these findings need to be confirmed in randomised and larger trials.

Interrupting or stopping antiplatelet therapy has been previously shown to associate with increased risk of cardiovascular events. Nevertheless, the impact

of interruption or stopping antiplatelet therapy in patients who have suffered ischaemic stroke is still lacking, thus, in chapter 5, the clinical outcomes following early cessation, interruption and stopping antiplatelet therapy were explored using a nested case-control study. In this observational study, early cessation or interrupted/stopped antiplatelet therapy was not associated with an increased risk of cardiovascular events compared to persistent users. The study used VISTA data, which has shorter study follow up (up to 90 days), could be one of the reasons the clinical outcomes could not be captured in this study. Further prospective and randomized trials are warranted to confirm these findings.

Cessation of DAPT may put patients at risk of cardiovascular event. Thus, in chapter 6 describes the incidence and predictors or cardiovascular events after DAPT cessation in patients who have suffered ACS using the NHS Greater Glasgow and Clyde Safe Haven database. The pertinent observations from this study were that: i) incidence of cardiovascular events occurred in 188 (16.7%) patients after DAPT cessation; ii) the incidence rate was lower in 0-90-day interval than in the next 90-day interval after DAPT cessation, 0.15 and 0.23 per 1000 patient-days, respectively; and iii) age, having medical or revascularization therapy and duration of DAPT were independent predictors of cardiovascular events. The evidence presented in this real life setting, may be found useful by clinician and cardiologist to guide DAPT cessation in their patients.

There are concerns regarding clustering of cardiovascular events that has been reported previously following clopidogrel cessation and it is essential to determine whether there are metabolite changes affected after DAPT cessation. In chapter 7, in patients who have suffered ACS, there were 311 putative metabolites identified after ticagrelor cessation. Of the 311 metabolites, 16 (5.1%) putative metabolites significantly altered following ticagrelor cessation, especially adenosine. Also, there were 19 putatively identified metabolites almost reach significance which requires further confirmation in larger sample. These putatively identified metabolites may have play a role in cardiovascular events following DAPT cessation that need to be investigated further.

The strength and limitations of each study in this thesis have been discussed in each individual chapter. Thus, in overall, the findings from the individual studies

described in this thesis raise important clinical questions that warrant further investigations. On the other hand, the conclusions obtain in relation to antiplatelet therapy and recurrent ischaemic stroke must be interpreted with caution. This is because the analyses were performed using a non-randomized clinical trial data from VISTA that were collated from numerous clinical trials. The study observed low events rate of recurrent stroke and lacks statistical power could be the reasons why the study may not detect significant difference between groups. Most analyses presented in this thesis were conducted retrospectively, thus need validation and further analyses using prospective studies.

8.1 Clinical implications

Ischaemic stroke and TIA are commonly complicated by increased risk of recurrence following acute event. Early prevention and multiple approaches could be beneficial for patients in order to improve disability following a stroke. The findings in this thesis suggested those who took antiplatelet therapy before a stroke, switching to a new antiplatelet regimen after ischaemic stroke might have a better functional outcome at 90 days. In patients who suffered ischaemic stroke, it is suggested appropriate and clinically guided interruptions to antiplatelet therapy may not put patients at significant increased risk within 90 days.

Discontinuation of DAPT is associated by increased risk of cardiovascular events. A better understanding of this may allow strategies to mitigate this risk. In this thesis, it is suggested to monitor closely ACS patients who is at risk i.e. elderly, those who received shorter DAPT duration (less than 6 months) and those who were not treated with revascularization. This evidence could be useful for clinicians in decision making or management of DAPT for their patients. Furthermore, these data suggest that ACS patients may benefit from longer duration of DAPT. Following DAPT cessation, there were no differences of cardiovascular event risk when continued either aspirin or clopidogrel mono antiplatelet therapy.

8.2 Future directions of research

Future directions include:

a) Long term clinical outcome data following ticagrelor cessation in patients who have suffered ACS are needed.

b) Metabolite changes between two time points could be compared before and after clopidogrel cessation in ACS patients.

c) Identification of novel biomarkers between patients with and without cardiovascular events following DAPT cessation could have potential implications.

Appendices

A1. Data security letter (VISTA)

Prof. Kennedy R. Lees, (VISTA Chair)
Institute of Cardiovascular and Medical Sciences
University of Glasgow
Dr. Myzoon Ali (VISTA Coordinator)
University of Glasgow
www.vistacollaboration.org
vista.acute@glasgow.ac.uk

Dear Dr. Dawson,

As requested, please find enclosed the dataset from VISTA. Contents are in the form of a zipped file to ensure the security of the data. The contents include demography, adverse event, medication tables and a data dictionary explaining labels and formats used. To activate the data please telephone +44 141 331 8121 for the unzip password.

This datasets will be sent to you on the condition that the data will remain the property of the VISTA Collaboration and no use will be made of it other than for the declared purpose. The data are confidential and therefore must not be disclosed to, or copied to, anyone else. Authorship must also be provided on behalf of the VISTA Collaboration.

At the end of this project, all copies of these data must be wiped from your computer or any other computer media and sources of back-up. For this purpose we assume that all data will have been removed from your system by 30th August 2014. If you wish to extend this date, we would be grateful if you could contact us regarding this. The project may be subject to an audit by the Robertson Center for Biostatistics upon completion.

If you agree to the above terms, please sign below and return this letter to me at the address below.

Yours Sincerely

Dr. Myzoon Ali
VISTA Coordinator
Division of Cardiovascular and Medical Sciences
Gardiner Institute
44 Church St.
Glasgow G11 6NT
Tel: 0141 331 8121
Fax: 0141 331 8101

I agree to the above terms and conditions:

Name.....
Date.....

Signature .....

A2. Keywords for stroke/TIA

Recurrent stroke	Transient ischaemic attack
2ND INFARCT IN HEAD OF CAUDATE NUCLEUS R	MULTIPLE TIA
A LARGE INFARCT IN ANTERIA CEREBRI MEDIA	RECURRENT T.I.A
ACUTE BRAIN INFARCT AT PONTINE LEVEL	RECURRING TRANSIENT ISCHEMIC ATTAACK
ACUTE ISCHEMIC STROKE	TIA
ACUTE ISCHEMIC STROKE (PROGRESSIVE)	TIA ATTACK
ACUTE LEFT CORTICAL INFARCT	TIA LEFT HEMISPHERE
ACUTE RIGHT CEREBROVASCULAR ACCIDENT	TRANS ISCHEMIC ATTACKS
ARTERIA CEREBRI MEDIA INFARCTION LEFT	TRANSIENT ISCHAEMIC ATTACK
ARTERIAL INTERNAL CAROTID ARTERY DISSECT	TRANSIENT ISCHAEMIC ATTACK
ARTERIAL INTERNAL CARTOID ARTERY DISSECT	TRANSIENT ISCHEMIC ATACK
ASYMPTOMATIC LEFT HEMISPHERIC INFARCTION	TRANSIENT ISCHEMIC ATTACK
BILATERAL ISCHEMIC STROKE	TRANSIENT ISCHEMIC ATTACK, MULTIPLE
BRAIN INFARCTION	TRANSIENT ISCHEMIC ATTACKS
BRAIN STEM INFARCTION	TRANSITORIC ISCHEMIC ATTACK
BRAIN STEM INFARCTION	TRANSITORY ISCHAEMIC ATTACK
CARDIOEMBOLIC MAJOR BRAIN STEM INFARCTIO	VERTEBROBASILAR TRANSIENT ISCHEMIC ATACK
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CEREBRAL RE-ISCHEMIA RIGHT HEMISPHERE	
CEREBRAL VASCULAR ACCIDENT	
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INFARCTION RT. MIDDLE CEREBRAL ARTERY	
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ISCHEMIC CEREBRAL INFARCTION	
ISCHEMIC STROKE	
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ISCHEMIC STROKE (FOLLOW UP)	
ISCHEMIC STROKE IN LEFT HEMISPHERE	
ISCHEMIC STROKE RECURRENCE	
LACUNAR INFARCTION	
LACUNAR STROKE	
LARGE LEFT CORTICAL INFARCT	
LARGE MALIGNANT INFARCT WITH MILD HEMORR	
LARGE RIGHT HEMISPHERIC STROKE	
LEFT BRAIN INFARCTION, NEW RESTROKE	
LEFT BRAIN INFARCTION, RESTROKE	
LEFT MIDDLE CEREBRAL ARTERY TERRITORY IN	
LEFT SIDED CEREBROVASCULAR ACCIDENT	
MALIGNANT CEREBRAL INFARCTION	
MALIGNANT INFARCTION	
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MALIGNANT RIGHT MCA INFARCT WITH EDEMA	
MASSIVE RIGHT CORTICAL HEMISPHERE INFARC	
MEDIA INFARCT LEFT SIDE	
MIDDLE CEREBRAL ARTERY INFARCT	
MIDDLE CEREBRAL ARTERY STROKE	
MULTIPLE ISCHEMIC STROKE	
MULTIPLE NEW STROKE	
MULTIPLE NEW STROKES - ISCHEMIC	
NEW ACUTE ISCHEMIC STROKE	
NEW BRAIN STEM CVA	
NEW BRAIN STROKE	
NEW BRAINSTEM STROKE	
NEW CEREBRAL INFARCTION	
NEW INFARCTION IN CONTRALATERAL HEMISPHE	
NEW ISCHAEMIC STROKE	
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NEW ISCHEMIC STROKE	
NEW ISCHEMIC STROKE #1	
NEW ISCHEMIC STROKE (RIGHT MIDDLE CEREBR	
NEW ISCHEMIC STROKE WITH HEMMORRHAGIC TR	
NEW ISCHEMIC STROKE WITH RIGHT HEMIPARES	

Recurrent stroke	Transient ischaemic attack
NEW ISCHEMIC STROKE, RIGHT SUBCORTICAL	
NEW LEFT ISCHEMIC CVA	
NEW ONSET ISCHEMIC STROKE	
NEW RIGHT CEREBELLAR INFARCTION	
NEW RIGHT ISCHEMIC CVA	
NEW STROKE	
NEW STROKE - ISCHEMIC	
NEW STROKE (BRAINSTEM)	
NEW STROKE (INFARCTION)	
NEW STROKE (ISCHEMIC)	
NEW STROKE HEMORRHAGIC	
NEW STROKE ISCHEMIC	
NEW STROKE LEFT BRAIN HAEMATOMA IN MCA T	
NEW STROKE LEFT OCCIPITAL ISCHEMIC STROK	
NEW STROKE SYMPTOMS IN REGIO CEREBELLARI	
NEW STROKE, ISCHAEMIC	
NEW STROKE/ISCHEMIC	
ORIGINAL LEFT MIDDLE CEREBRAL ARTERY INF	
ORIGIONAL ACUTE ISCHEMIC STROKE	
REC. STROKE	
RECIDIVE BRAIN INFARCTION	
RECURREND STROKE	
RECURRENT ACUTE ISCHEMIC STROKE - CEREBE	
RECURRENT CEREBRAL ISCHEMIA	
RECURRENT CEREBROVASCULAR ACCIDENT	
RECURRENT IPSILATERAL STROKE	
RECURRENT ISCHAEMIC NEW STROKE LEFT HEMI	
RECURRENT ISCHAEMIC STROKE	
RECURRENT ISCHEMIC CEREBROVASCULAR STROK	
RECURRENT ISCHEMIC STROKE	
RECURRENT ISCHEMIC STROKE - RIGHT POSTER	
RECURRENT LEFT HEMISPHERIC STROKE	
RECURRENT NEW ISCHEMIC STROKE	
RECURRENT RIGHT MCA STROKE	
RECURRENT STROKE	
RECURRENT STROKE (FOLLOW UP)	
RE-INFARCTION OF LEFT LENTIFORM NUCLEUS	
RE-STROKE ISCHEMIC	
RIGHT CEREBRAL INFARCT	
RIGHT MIDDLE CEREBRAL ARTERY STROKE	
RIGHT PARIETAL ISCHEMIC INFARCT	
RT. MCA INFARCT WITH MASSIVE CEREBRAL ED	
STROKE	
STROKE (ACV)	
STROKE (ISCHAEMIC)	
STROKE (ISCHEMIC)	
STROKE (LEFT MIDDLE CEREBRAL ART.)	
STROKE (NEW EVENT)	
STROKE (NEW)	
STROKE (RECURRENCE)	
STROKE BRAINSTEM	
STROKE IN RIGHT ANTERIOR AND MIDDLE CERE	
STROKE RECURRENCE	

A3. Keywords for bleeding

Intracranial	Extracranial (gastrointestinal, non-gastrointestinal)
BILATERAL SUBDURAL HEMATOMA	ANAEMIA DUE TO GASTROINTESTINAL BLOOD LO
ACUTE HEMORRHAGIC STROKE	BLEEDING DUODENAL ULCER
AND SUBARACHNOID HEMORRHAGE	BLEEDING FROM THE ULCER IN STOMACH
ASYMPTOMATIC CEREBRAL HAEMORRHAGIC TRANS	BLEEDING GASTRIC ULCER
ASYMPTOMATIC HAEMORRHAGIC INFARCTION (ON	BLEEDING GASTRIC ULCERS
ASYMPTOMATIC INTRACRANIAL BLEEDING	BLEEDING GASTROINTESTINAL TRACT
ASYMPTOMATIC MILD INTRA-CEREBRAL BLEEDIN	BLEEDING IN ABDOMINAL LOCALITY WITH ABDO
ASYMTOMATIC CEREBRAL BLEEDING	BLOOD CLOT IN URINE
BASAL GANGELION HAEMORRHAGE	BLOOD IN STOOL
BLEEDING INTRACRANIAL	BLOOD IN URINE
BRAIN HAEMORRHAGE	BLOOD IN URINE
BRAIN HEMATOMA	BLOOD VOMITING
BRAIN HEMATOMA INTRAVENTRICULAR HEMORRHA	BLOODY STOOL
BRAIN HEMATOMA WITH INTRAVENTRICULAR HEM	COFFEE GROUND EMESIS
BRAIN HEMATOMA WITH SUBARACHNOID HEMORRH	COFFEE GROUND VOMITING
BRAIN HEMORRHAGE	DUODENAL HEMORRHAGE
CEREBELLAR HAEMORRHAGE (ARTEFACT?) REPOR	ENTERORHAGY – INTESTINAL HAEMORRHAGE
CEREBELLAR HEMORRHAGE	FRANK HAEMATURIA
CEREBRAL BLEEDING	GASTRIC BLEEDING
CEREBRAL HAEMORRAGE	GASTRIC HAEMORRHAGE
CEREBRAL HAEMORRHAGE	GASTRIC HEMORRHAGE
CEREBRAL HEMATOMA	GASTRIC ULCER WITH SERIOUS BLEEDING
CEREBRAL HAEMATOMA	GASTRO DUODENAL BLEEDING
CEREBRAL HEMORRAGE	GASTRO INTESTINAL BLEED
CEREBRAL HEMORRHAGE	GASTRO INTESTINAL BLEEDING
CEREBRAL HEMORRHAGE	GASTROINTESTINAL BLEED
CEREBRAL HEMORRHAGE (FOLLOW-UP)	GASTROINTESTINAL BLEED SECONDARY TO GAST
CEREBRAL HEMORRHAGE EVENT	GASTROINTESTINAL BLEEDING
CEREBRAL HEMORRHAGY	GASTROINTESTINAL HAEMORRAGE
CEREBRAL HAEMORRHAGE	HAEMORRHAGE OF RECTUM
HAEMATOMA SUBDURAL	GASTROINTESTINAL HAEMORRHAGE
HAEMORRHAGE (INTRACRANIAL)	GASTROINTESTINAL HEMORRHAGE
HAEMORRHAGE BRAIN	GASTROINTESTINAL TRACT BLEED NOS
HAEMORRHAGE INTO CEREBRAL INFARCTION	GI BLEED
HAEMORRHAGE INTO ORIGINAL INFARCT	GI BLEEDING
HAEMORRHAGE INTRACRANIAL	GI HAEMORRHAGE
HAEMORRHAGIC CEREB.INFARCT.IN PRIMARY FO	GROSS HEMATURIA
HAEMORRHAGIC CEREBRAL INFARCTION	MASSIVE EPISTAXIS
HAEMORRHAGIC STROKE	MASSIVE EPISTAXIS WITH HEMORRHAGIC SHOCK
HEMMORHAGIC STROKE	HAEMATEMESIS
HEMORRHAGE (CEREBRAL)	HAEMATURIA
HEMORRHAGE (INTRACRANIAL)	HAEMORRHAGE FROM DIGESTIVE TRACT
HEMORRHAGE BRAIN	HAEMORRHAGE FROM THE URINARY TRACT
HEMORRHAGE CEREBRAL	HAEMORRHAGE GASTROINTESTINAL
HEMORRHAGE IN STROKE TERRITORY BY MRI	HAEMORRHAGE URINARY TRACT
HEMORRHAGE INTRACEREBRAL	HEMATOCHEZIA
HEMORRHAGE INTRACRANIAL	HEMATOCHEZIA – STOOLS WITH BLOOD
HEMORRHAGE LEFT BASAL GANGLIA	HEMATURIA
HEMORRHAGE LEFT FRONTAL LOBE	HEMATURIA AGGRAVATED
HEMORRHAGE WITHIN THE ISCHAEMIC TERRITOR	HEMATURIA TRAUMATIC
HEMORRHAGE, CEREBRAL	HEMORRHAGE OF GASTROINTESTINAL TRACT
HEMORRHAGE, ENLARGEMENT OF INFARCTED ARE	HEMORRHAGIC SHOCK
HEMORRHAGIC STROKE	INTESTINAL BLEEDING
HEMORRHAGIC STROKE (HEMORRHAGICAL TRANSF	INTESTINAL HAEMORRHAGE
I.C. HAEMORRHAGES	INTRA-ABDOMINAL HEMORRHAGE
IC HAEMORRHAGIA DX WITH SLIGHT CLINICAL	RECTAL BLEED
ICH (INTRACRANIAL CEREBRAL HEMORRHAGE)	RECTAL BLEEDING
INTRA CEREBRAL HAEMORRHAGE	RECTAL BLOOD POST BOWEL MOVEMENT SEC TO
INTRA CEREBRAL HEMORRHAGE	RECTAL HAEMORRHAGE
INTRA CRANIAL BLEED	RECTAL HEMORRHAGE
INTRA VENTRICULAR HEMORRHAGE WITH CLINIC	HEMORRHAGE OF RECTUM AND ANUS
INTRACEBRAL BLEEDING	LOWER GASTROINTESTINAL BLEED
INTRACEREBELLAR HAEMORHAGY	LOWER GASTROINTESTINAL HEMORRHAGE
INTRACEREBRA HEMORRHAGE TYPE 2	LOWER GI BLEED
INTRACEREBRAL & VENTRICULAR BLEEDING	MELAENA
INTRACEREBRAL BLEED	MELAENA
INTRACEREBRAL BLEEDING	MELAENAS
INTRACEREBRAL BLEEDING AFTER TROMBOLYSIS	MELENA
INTRACEREBRAL HAEMATOMA	MELENA
INTRACEREBRAL HAEMORRAGE	MELENA DUE TO DUODENAL ULCER

Intracranial	Extracranial (gastrointestinal, non-gastrointestinal)
INTRACEREBRAL HAEMORRAGHE	HEMORRHAGE OF RECTUM
INTRACEREBRAL HAEMORRHAGE	OESOPHAGEAL BLEEDING
INTRA-CEREBRAL HAEMORRHAGE	PEPTIC ULCER HAEMORRHAGE
INTRACEREBRAL HAEMORRHAGE (FOLLOW-UP)	PEPTIC ULCER WITH HAEMORRHAGE
INTRACEREBRAL HAEMORRHAGE INTO ACUTE INF	PEPTIC ULCER WITH HAEMORRHAGY
INTRACEREBRAL HEMMORRHAGE	RETENTION OF CLOTS (HAEMATURIA-BLOOD IN
INTRACEREBRAL HEMORRAGE NEW STROKE	SEVERE GASTROINTESTINAL BLEEDING LEADING
INTRACEREBRAL HEMORRHAGE	SHOCK DUE TO GASTROINTESTINAL BLEEDING
INTRA-CEREBRAL HEMORRHAGE	STOMACH ULCER HEMORRHAGE
INTRACEREBRAL HEMORRHAGE (NEW)	TARRY STOOLS
INTRACEREBRAL HEMORRHAGE ASYMPTOMATIC (T	UNSPECIFIED GASTRIC (STOMACH) HEMORRHAGE
INTRACEREBRAL HEMORRHAGE MALIGNANT OEDEM	UPPER GASTRO INTESTINAL BLEEDING
INTRACEREBRAL HEMORRHAGE S/P FALL	UPPER GASTROINTESTINAL BLEED
INTRACRANIAL BLEEDING	UPPER GASTROINTESTINAL BLEEDING
INTRACRANIAL BLEEDING	UPPER GASTROINTESTINAL BLEEDING SECONDAR
INTRACRANIAL HAEMATOMA	UPPER GASTROINTESTINAL HAEMORRHAGE
INTRACRANIAL HAEMORRHAGE	UPPER GASTROINTESTINAL HEMORRHAGE
INTRACRANIAL HAEMORRHAGE NOS	UPPER GI BLEED
INTRACRANIAL HEMATOMA	UPPER GI HEMORRHAGE ^/= HEMATEMESIS
INTRACRANIAL HEMORRAGE	VOMITING BLOOD
INTRACRANIAL HEMORRHAGE	WITH MACRO HEMATURIA
INTRACRANIAL HEMORRHAGE, UNSPECIFIED	HEMORRHAGE
INTRAVENTRICULAR HEMORRHAGE	MALLORY WEISS SYNDROME
LEFT CAPSULA INTERNA BLEEDING	MALLORY WEISS TEAR
LEFT INTRACEREBRAL HEMORRHAGE (LARGE)	MULTIPLE GASTRIC ULCERS WITH HEMORRHAGE
MASSIVE RIGHT INTRACEREBRAL HEMORRHAGE	
MINOR CEREBRAL BLEED RELATED TO BRAIN BI	
MINOR INTRACEREBRAL HAEMORRHAGE	
NEW HEMMORRHAGIC STROKE	
NEW HEMORRHAGIC STROKE	
NEW INTRACEREBRAL HEMORRHAGE	
PETECHIAL HAEMORRHAGE OF INF. AREA	
PETECHIAL INTRACEREBRAL BLEEDING, ASYMPT	
PETECHIAL INTRACEREBRAL HEMATOMA, ASYMPT	
POST TPA CEREBRAL PARENCHYMAL HEMORRHAGE	
PRIMARY INTRA-CEREBRAL HAEMORRHAGE	
RECURRENT HEMORRHAGIC STROKE	
REPERFUSION HEMORRHAGE OF BRAIN	
RIGHT SIDED INTRACRANIAL HAEMORRHAGE	
SECONDARY HEMORRHAGE IN INFARCTED AREA	
SEVERE HAEMORRHAGIC STROKE	
SMALL RIGHT FRONTAL CEREBRAL HEMORRHAGE	
SUB DURAL HAEMATOMA	
SUBARACHNOID HAEMORRHAGE	
SUBARACHNOID HEMORRHAGE	
SUBDURAL HAEMATOMA	
SUBDURAL HEMATOMA	
SUBDURAL HEMATOMA (TRAUMATIC)	
SUBDURAL HEMORRHAGE	
SUBDURAL HEMORRHAGE, BILATERAL	
SPINAL EPIDURAL HEMATOMA	
SYMPTOMATIC INTRACRANIAL HEMORRHAGE	
THALAMUS HAEMATOMA	
TRAUMATIC SUBDURAL HEMATOMA	
EPIDURAL HAEMATOMA	
HEMORRHAGISATION OF STROKE IN TERRITORRY	

A4. Safe Haven application feedback

NHSGGC Safe Haven

Safe Haven
Health Research Informatics Unit
Level 6, Boyd Orr Building
University of Glasgow
University Avenue
Glasgow
G12 8QQ

Date: 13.02.14

Ms. Wardati Mazlan-Kepli
Institute of Cardiovascular and Medical Sciences
College of Medical, Veterinary & Life Sciences
University of Glasgow
Gardiner Institute, Western Infirmary
Glasgow
G11 6NT

Safe Haven Application Feedback

Project Name: **Antiplatelet therapy and clinical outcomes in cardiovascular patients**

Project Number: **GSH/13/CA/005**

All applications to the Safe Haven are reviewed separately by the Safe Haven team, the R&D Peer Review Committee (if appropriate) and the LPAC group and measured against a set of pre-defined criteria.

Your application has been through all relevant review processes and found to be generally acceptable; the committee has issued a **conditional approval**, provided you amend the following aspects of your study:

- Please provide more specific information on what data fields are required. It was agreed that this project could be used as a pilot project for using GJNH data.
- It is suggested that you work on questions 1, 3 and 4 first. The Safe Haven does not currently hold the data to answer question 2 'Are there any clinical predictors of cardiovascular events following cessation of DAPT?', but this data should be available later, as a result of another study currently underway.

NHSGGC Safe Haven

Please submit your amendments in writing to a member of the Safe Haven team, who will confirm that your application is complete and approved.

We look forward to working with you and thank you for considering the Safe Haven to be part of your study.

Yours Sincerely

C.MacDonald

Claire MacDonald
Safe Haven Project Manager

A5. ICD-10 codes for cardiovascular events

Events	ICD-10 codes	Diseases
ACS	I20.0	Unstable angina
	I21	Acute myocardial infarction
	I21.0	Acute transmural myocardial infarction of anterior wall
	I21.1	Acute transmural myocardial infarction of inferior wall
	I21.2	Acute transmural myocardial infarction of other sites
	I21.3	Acute transmural myocardial infarction of unspecified site
	I21.4	Acute subendocardial myocardial infarction
	I21.9	Acute myocardial infarction, unspecified
	I22	Subsequent myocardial infarction
	I22.0	Subsequent myocardial infarction of anterior wall
	I22.1	Subsequent myocardial infarction of inferior wall
	I22.8	Subsequent myocardial infarction of other sites
	I22.9	Subsequent myocardial infarction of unspecified site
Heart failure	I50	Heart failure
TIA	G45.8	Other transient cerebral ischaemic attacks and related syndromes
	G45.9	Transient cerebral ischaemic attack, unspecified
Stroke	I63	Cerebral infarction
	I63.0	Cerebral infarction due to thrombosis of precerebral arteries
	I63.1	Cerebral infarction due to embolism of precerebral arteries
	I63.2	Cerebral infarction due to unspecified occlusion or stenosis of precerebral arteries
	I63.3	Cerebral infarction due to thrombosis of cerebral arteries
	I63.4	Cerebral infarction due to embolism of cerebral arteries
	I63.5	Cerebral infarction due to unspecified occlusion or stenosis of cerebral arteries
	I63.6	Cerebral infarction due to cerebral venous thrombosis, nonpyogenic
	I63.8	Other cerebral infarction
	I63.9	Cerebral infarction, unspecified
	I64	Stroke, not specified as haemorrhage or infarction
Cardiac death	I46.1	Sudden cardiac death

A6. ICD-10 codes for major bleeding

ICD-10 codes	Diseases
Subarachnoid	
I60	Subarachnoid haemorrhage
I60.0	Subarachnoid haemorrhage from carotid siphon and bifurcation
I60.1	Subarachnoid haemorrhage from middle cerebral artery
I60.2	Subarachnoid haemorrhage from anterior communicating artery
I60.3	Subarachnoid haemorrhage from posterior communicating artery
I60.4	Subarachnoid haemorrhage from basilar artery
I60.5	Subarachnoid haemorrhage from vertebral artery
I60.6	Subarachnoid haemorrhage from other intracranial arteries
I60.7	Subarachnoid haemorrhage from intracranial artery, unspecified
I60.8	Other subarachnoid haemorrhage
I60.9	Subarachnoid haemorrhage, unspecified
Intracerebral	
I61	Intracerebral haemorrhage
I61.0	Intracerebral haemorrhage in hemisphere, subcortical
I61.1	Intracerebral haemorrhage in hemisphere, cortical
I61.2	Intracerebral haemorrhage in hemisphere, unspecified
I61.3	Intracerebral haemorrhage in brain stem
I61.4	Intracerebral haemorrhage in cerebellum
I61.5	Intracerebral haemorrhage, intraventricular
I61.6	Intracerebral haemorrhage, multiple localized
I61.8	Other intracerebral haemorrhage
I61.9	Intracerebral haemorrhage, unspecified
Others	
I62	Other nontraumatic intracranial haemorrhage
I62.0	Subdural haemorrhage (acute)(nontraumatic)
I62.1	Nontraumatic extradural haemorrhage
I62.9	Intracranial haemorrhage (nontraumatic), unspecified
Intraocular	
H11.3	Conjunctival haemorrhage (Subconjunctival harmorrhage)
H31.3	Choroidal haemorrhage and rupture
H35.6	Retinal haemorrhage
H43.1	Vitreous haemorrhage
Retroperitoneal	
R58	Haemorrhage, not elsewhere classified

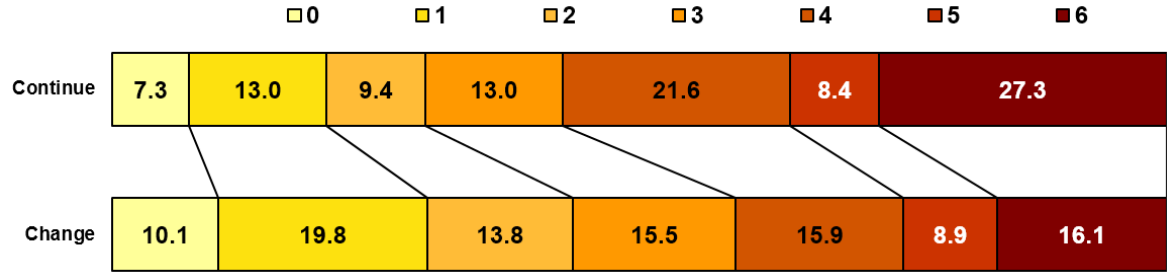
A7. Additional analyses (Chapter 3)

a) Clinical outcomes at 90 days

Outcomes	Change n=538	Continue n=591	Adjusted OR* (95% CI)	p-value
Recurrent stroke	22/532 (4.1)	25/588 (4.3)	1.35 (0.70-2.60)	0.367
ICH	13/532 (2.4)	15/581 (2.6)	0.98 (0.40-2.41)	0.957
ECH	25/527 (4.7)	17/584 (2.9)	2.03 (1.04-3.98)	0.039

All values are reported as no. (%) unless otherwise noted. *All models are adjusted for age, baseline NIHSS, hypertension, AF, prior TIA, prior stroke and rt-PA. AF= atrial fibrillation, CI=confidence interval, ICH=intracranial haemorrhage, ECH=extracranial haemorrhage, OR=odds ratio, rt-PA=recombinant tissue plasminogen activator, TIA= transient ischaemic attack.

b) Distribution of mRS outcome at day 90 in patients suffering ischaemic stroke (change vs continue group)



Adjusted OR, 1.47; 95% CI, 1.11-1.83; p=0.002*

Diagram showing association of functional outcome at day 90 between change and continue group. *Model is adjusted for age, baseline NIHSS, hypertension, AF, prior TIA, prior stroke and rt-PA. AF= atrial fibrillation, CI=confidence interval, OR=odds ratio, rt-PA=recombinant tissue plasminogen activator, TIA= transient ischaemic attack. Values provided in each box denote the percentage of patients belonging to a specific treatment category (change or continue) and representing the mRS score corresponding to the box.

A8. Multiple variables matching using SAS software

```
%LET AGERANGE = 10;
%LET RATIO = 4;
DATA CASES CONTROLS;
SET All;
IF EVENT = 0 THEN OUTPUT CASES;
ELSE OUTPUT CONTROLS;
PROC FREQ NOPRINT DATA=CASES;
TABLES AGE*DIABETES/OUT=CASEOUT;

%MACRO SAMPLE
(V_AGE,V_RESPONSE,V_COUNT);
DATA QUALIFY1; SET CONTROLS;
WHERE (&V_AGE-&AGERANGE
<=AGE<=&V_AGE+&AGERANGE)
AND (DIABETES = "&V_RESPONSE");

CASES_AGE=&V_AGE;
CASES_DIABETES="&V_DIABETES";

SEED=RANUNI(0);
PROC SORT; BY SEED;

DATA QUALIFY2;
SET QUALIFY1 NOBS=TOTOBS;
IF _N_<=&V_COUNT*&RATIO;
IF &V_COUNT*&RATIO <= TOTOBS THEN TAG = 'YES';
ELSE TAG = 'NO';

PROC APPEND BASE=MATCHES DATA=QUALIFY2;

PROC SORT DATA=QUALIFY2 OUT=TEMP1
(KEEP=NUMBER); BY NUMBER;

PROC SORT DATA=CONTROLS OUT=TEMP2;
BY NUMBER;

DATA CONTROLS;
MERGE TEMP1(IN=IN1) TEMP2(IN=IN2);
BY NUMBER; IF IN2 AND NOT IN1;

%MEND SAMPLE;
```

A9. REC feedback letter

NHS
Health Research Authority
National Research Ethics Service

NRES Committee North West - Greater Manchester East
3rd Floor, Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7820

28 July 2014

Dr Jesse Dawson, Clinical Senior Lecturer in Medicine and Clinical Pharmacology
Cardiovascular and Med. Science
Western Infirmary
Glasgow
G11 6NT

Dear Dr Dawson

Study title:	**Stopping dual anti-platelet treatment following acute coronary syndrome; a metabolic analysis.**
REC reference:	**14/NW/1163**
Protocol number:	**1**
IRAS project ID:	**158423**

The Proportionate Review Sub-committee of the NRES Committee North West - Greater Manchester East reviewed the above application on 28 July 2014.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Elaine Hutchings, nrescommittee.northwest-gmeast@nhs.net.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study:

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Additional condition

- The logos should appear consistently on the supporting documentation (i.e. both logos should appear on all documents and in the same order) and should appear at the top of each document rather than sometimes being placed at the bottom.

Please notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Registration of Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Summary of discussion at the meeting

This study was found to be well thought through with a good recruitment and opt-in process. It presented no major issues. The risks involved were minimal and patients' care is good with transport by taxi being provided.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Advertisement]	1	03 July 2014
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		30 July 2013
GP/consultant information sheets or letters	1	25 June 2014
IRAS Checklist XML		16 July 2014
Letters of invitation to participant	1	25 June 2014
Other [Case Report Form]	1	12 June 2014
Participant consent form	1	12 June 2014
Participant information sheet (PIS)	1	12 June 2014
REC Application Form [158423/639349/1/582]		16 July 2014
Research protocol or project proposal [Research Protocol]	1	01 June 2014
Summary CV for Chief Investigator (CI) [Dr Jesse Dawson]		29 April 2013
Summary CV for student [Ms Wardati Mazlan Kepli]		23 April 2014
Summary CV for supervisor (student research) [Prof Matthew Walters]		
Validated questionnaire [Morisky scale]	1	12 June 2014

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

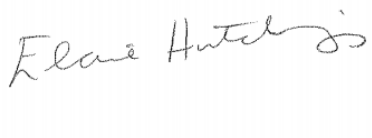
You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

14/NW/1163	**Please quote this number on all correspondence**

Yours sincerely



pp
Mr Francis Chan
Chair

Email: nrescommittee.northwest-gmeast@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

"After ethical review – guidance for researchers"

Copy to: *Emma-Jane Gault, NHS Greater Glasgow and Clyde*

Dr Erica Packard, NHS Greater Glasgow and Clyde

NRES Committee North West - Greater Manchester East

Members of the Committee who took part in the review

Committee Members:

Name	*Profession*	*Present*	*Notes*
Mr James Burns	Retired	Yes	
Mr Christopher Houston	Lay Member	Yes	
Mr Simon Jones	Specialist Podiatrist - Paediatrics	Yes	
Professor Janet Marsden	Professor of Ophthalmology and Emergency Care	Yes	Chairing

Also in attendance:

Name	*Position (or reason for attending)*
Elaine Hutchings	REC Manager

A10. REC documents approval

NHS
Health Research Authority
National Research Ethics Service

NRES Committee North West - Greater Manchester East
3rd Floor, Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7820

14 August 2014

Dr Jesse Dawson, Clinical Senior Lecturer in Medicine and Clinical Pharmacology
Cardiovascular and Med. Science
Western Infirmary
Glasgow
G11 6NT

Dear Dr Dawson

Study title: **Stopping dual anti-platelet treatment following acute coronary syndrome; a metabolic analysis.**
REC reference: **14/NW/1163**
Protocol number: **1**
IRAS project ID: **158423**

Thank you for your letter of 8 August 2014. I confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 28 July 2014

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Advertisement]	2	07 August 2014
Validated questionnaire [Morisky Scale]	2	07 August 2014
GP/consultant information sheets or letters	2	07 August 2014
Other (Case report form)	2	07 August 2014
Letters of invitation to participant	2	07 August 2014

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Advertisement]	2	07 August 2014
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		30 July 2013
GP/consultant information sheets or letters	2	07 August 2014
IRAS Checklist XML		16 July 2014
Validated questionnaire [Morisky Scale]	2	07 August 2014
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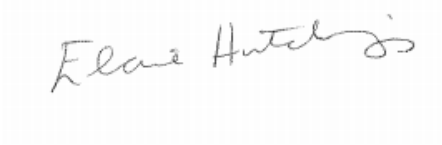
Summary CV for student [Ms Wardati Mazlan Kepli]		23 April 2014
Summary CV for supervisor (student research) [Prof Matthew Walters]		
Validated questionnaire [Morisky Scale]	2	07 August 2014

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

14/NW/1163 **Please quote this number on all correspondence**

Yours sincerely



Elaine Hutchings
REC Manager

E-mail: nrescommittee.northwest-gmeast@nhs.net

Copy to: *Dr Jesse Dawson, University of Glasgow*

Emma-Jane Gault, NHS Greater Glasgow and Clyde

Dr Erica Packard, NHS Greater Glasgow and Clyde

Wardati Mazlan-Kepli

A11. Letter of access for research

Coordinator/Administrator: Dr Erica Packard/Mrs Elaine O'Neill
Telephone Number: 0141 232 9448
E-Mail: erica.packard@ggc.scot.nhs.uk
Website: www.nhsggc.org.uk/r&d

R&D Management Office
Western Infirmary
Tennent Building
1st Floor, 38 Church Street
Glasgow, G11 6NT.

12 August 2014

Ms Wardati Mazlan Kepli
Institute of Cardiovascular and Medical Sciences
Western Infirmary
Dumbarton Road
Glasgow G11 6NT

Dear Ms Mazlan Kepli,

Letter of Access for Research

This letter confirms your right of access to conduct research through **NHS Greater Glasgow and Clyde** for the purpose and on the terms and conditions set out below. This right of access commences on **12/08/2014** and ends on **12/10/2015** unless terminated earlier in accordance with the clauses below.

You have a right of access to conduct such research as confirmed in writing in the letter of permission for research from this NHS organisation. Please note that you cannot start the research until the Principal Investigator for the research project has received a letter from us giving permission to conduct the project.

The information supplied about your role in research at **NHS Greater Glasgow and Clyde** has been reviewed and you do not require an honorary research contract with this NHS organisation. We are satisfied that such pre-engagement checks as we consider necessary have been carried out.

You are considered to be a legal visitor to **NHS Greater Glasgow and Clyde** premises. You are not entitled to any form of payment or access to other benefits provided by this NHS organisation to employees and this letter does not give rise to any other relationship between you and this NHS organisation, in particular that of an employee.

While undertaking research through **NHS Greater Glasgow and Clyde**, you will remain accountable to **the University of Glasgow** but you are required to follow the reasonable instructions of **Dr Jesse Dawson** in this NHS organisation or those given on her/his behalf in relation to the terms of this right of access.

Where any third party claim is made, whether or not legal proceedings are issued, arising out of or in connection with your right of access, you are required to co-operate fully with any investigation by this NHS organisation in connection with any such claim and to give all such assistance as may reasonably be required regarding the conduct of any legal proceedings.

You must act in accordance with **NHS Greater Glasgow and Clyde** policies and procedures, which are available to you upon request, and the Research Governance Framework.

You are required to co-operate with **NHS Greater Glasgow and Clyde** in discharging its duties under the Health and Safety at Work etc Act 1974 and other health and safety legislation and to take reasonable care for the health and safety of yourself and others while on **NHS Greater Glasgow and Clyde** premises. You must observe the same standards of care and propriety in dealing with patients, staff, visitors, equipment

and premises as is expected of any other contract holder and you must act appropriately, responsibly and professionally at all times.

If you have a physical or mental health condition or disability which may affect your research role and which might require special adjustments to your role, if you have not already done so, you must notify your employer and the health board's HR department prior to commencing your research role at the Health board.

You are required to ensure that all information regarding patients or staff remains secure and *strictly confidential* at all times. You must ensure that you understand and comply with the requirements of the NHS Confidentiality Code of Practice (http://www.dh.gov.uk/assetRoot/04/06/92/54/04069254.pdf) and the Data Protection Act 1998. Furthermore you should be aware that under the Act, unauthorised disclosure of information is an offence and such disclosures may lead to prosecution.

You should ensure that, where you are issued with an identity or security card, a bleep number, email or library account, keys or protective clothing, these are returned upon termination of this arrangement. Please also ensure that while on the premises you wear your ID badge at all times, or are able to prove your identity if challenged. Please note that this NHS organisation accepts no responsibility for damage to or loss of personal property.

We may terminate your right to attend at any time either by giving seven days' written notice to you or immediately without any notice if you are in breach of any of the terms or conditions described in this letter or if you commit any act that we reasonably consider to amount to serious misconduct or to be disruptive and/or prejudicial to the interests and/or business of this NHS organisation or if you are convicted of any criminal offence. You must not undertake regulated activity if you are barred from such work. If you are barred from working with adults or children this letter of access is immediately terminated. Your employer will immediately withdraw you from undertaking this or any other regulated activity and you MUST stop undertaking any regulated activity immediately.

Your substantive employer is responsible for your conduct during this research project and may in the circumstances described above instigate disciplinary action against you.

NHS Greater Glasgow and Clyde will not indemnify you against any liability incurred as a result of any breach of confidentiality or breach of the Data Protection Act 1998. Any breach of the Data Protection Act 1998 may result in legal action against you and/or your substantive employer.

If your current role or involvement in research changes, or any of the information provided in your Research Passport changes, you must inform your employer through their normal procedures. You must also inform your nominated manager in this NHS organisation.

Yours sincerely

EPackard

Dr Erica Packard
Research Co-ordinator

cc: **Dr Debra Stuart (University of Glasgow)**

A12. Advertisement

DUAL ANTI-PLATELET TREATMENT FOLLOWING ACUTE CORONARY SYNDROME - A METABOLIC ANALYSIS

Have you suffered a heart attack?

Are you taking any two of these blood thinning drugs?

- **Aspirin**
- **Clopidogrel**
- **Ticagrelor**

If so then you may be eligible for a new research study. The study aims to help us better understand what happens in the blood when we change blood thinning drugs after heart problems.

What's involved?

The study involves 2 visits to the Acute Stroke Unit, Western Infirmary. The study will involve 2 blood and urine samples being taken and a telephone call.

For more information please contact

Ms Wardati Mazlan-Kepli or Dr Jesse Dawson

Institute Cardiovascular and Medical Sciences

Western Infirmary

Glasgow

G11 6NT

E-mail: w.mazlan-kepli.1@research.gla.ac.uk

Telephone: 074 4811 4659

A13. Letter to general practices

Dr *<GP Name>*
Address line 1
Address line 2
Address line 3

Dear Dr

Re: Patient ..... D.O.B. .....

Your patient, named above, has given their consent to enrol in the Stopping Dual Antiplatelet Treatment (DAPT) Following Acute Coronary Syndrome research study.

This is a prospective study which aims to identify metabolomic changes that occur when patients switch from DAPT to anti-platelet monotherapy after an acute coronary syndrome. Metabolomics is the study of metabolite levels in a biologic sample and allows assessment of all identifiable metabolites simultaneously.

Participants will be asked to attend the Acute Stroke Unit at the Western Infirmary twice; to give a blood and urine sample within the month before and the month after their planned DAPT stop date. The study itself will not interfere with the DAPT stop date or their medication in any way.

If you require any further information about the Stopping DAPT After ACS study, please either contact me or Dr Jesse Dawson, Chief Investigator of the study at the University of Glasgow Gardiner Institute / Western Infirmary Stroke Unit.

Many thanks,

Wardati Mazlan Kepli
PhD Student
Email: w.mazlan-kepli.1@research.gla.ac.uk
Tel: 074 4811 4659

PhD Office
Gardiner Institute
University of Glasgow
Western Infirmary
Glasgow G11 6NT

A14. Letter to potential participants

Dear <*Mr. / Ms. LAST NAME*>,

I am writing to let you know about a research study taking place in the Western Infirmary, Glasgow. The study is called "Stopping dual anti-platelet treatment following an acute coronary syndrome." It is being conducted by Ms Wardati Mazlan-Kepli, PhD Student and Dr Jesse Dawson, Clinical Senior Lecturer in Medicine based in the Acute Stroke Unit, Western Infirmary and the University of Glasgow.

I am writing to you because from our records show you have suffered an acute coronary syndrome and that you have been prescribed two blood thinning drugs. It is normal after a period of time for one of these two blood thinning drugs to stop and for you to continue on just one. The researchers want to see whether this leads to detectable changes in the blood and urine using some new laboratory techniques.

In the study, the researchers will take a blood and urine sample before and shortly after the date that you change from two blood thinning tablet to one (this date is decided by your cardiologist). This will involve two short trips to the Western Infirmary.

This letter serves only to inform you about the study. You do not need to take part and deciding not to participate will not affect your care. However, if you are interested in learning more, please let the researchers know and they will contact you. You can do this by returning the attached form or by contacting Ms Mazlan-Kepli by telephone on 074 4811 4659. You do not have to respond if you are not interested in this study.

Thank you for your time and consideration. The researchers look forward to hearing from you.

Yours sincerely,

FROM CARE PROVIDER

***Include enclosure(s) as applicable:**
Opt-in Form
Participant information sheet
Advertisement

OPT-IN FORM

Stopping dual anti-platelet treatment following acute coronary syndrome;

A metabolic analysis

Please complete this form and return in the pre-paid envelope provided

☐ I am interested in learning more about this study. Please contact me using the following information:

Name : _____

Telephone(s) : _____

Best time and day to call : _____

Email : _____

Please return this form in the pre-paid envelope provided, or to:

Ms Wardati Mazlan-Kepli
Institute of Cardiovascular and Medical Sciences
Gardiner Institute
University of Glasgow
G11 6NT

A15. Participant information sheets

THIS SHEET HAS BEEN APPROVED BY THE WEST OF SCOTLAND RESEARCH ETHICS SERVICES

PARTICIPANT INFORMATION SHEET FOR PARTICIPANTS IN A CLINICAL RESEARCH PROJECT

Title of Project

Stopping dual anti-platelet treatment following acute coronary syndrome; A metabolic analysis

You are being invited to take part in a clinical research study. This study aims to help us better understand what happens in the blood when we change blood thinning drugs after heart problems. The study is part of a PhD being performed in the University of Glasgow. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. The first part of the form explains what will happen if you decide to participate and the second explains important information about what will happen to the data we generate in the study and your rights as a participant.

Thank you for reading this.

Name of Researchers

Ms W Mazlan-Kepli, Phd Student
Dr J Dawson, Clinical Senior Lecturer in Medicine (Supervisor)
Prof M Walters, Professor of Clinical Pharmacology

Purpose of the Study

You have been diagnosed as having suffered a heart attack or bad angina episode in the past. Following this you were taking two blood thinning drugs (called anti-platelets) to reduce the risk of further problems. Usually this is a combination of aspirin and another drug called either clopidogrel or ticagrelor. These two drugs help keep platelets in the blood from sticking together and forming clots. However, after a period of time the two drugs are no longer needed so and patients then take only one anti-platelet drug (usually aspirin). We want to see what happens in the blood of patients when they switch to taking only one anti-platelet drug. We want to do this as sometimes when people stop the two drugs there is a risk of blood clots recurring. This is very unusual but we want to see if we can work out why.

In this study we plan to follow a series of patients with recent heart problems and measure levels of small molecules called metabolites in the blood before and after they switch from two antiplatelets to one. This preliminary study may allow us to identify changes that might predict future blood clot events that would warrant further study.

Why Have I Been Chosen?

All patients who are taking two anti-platelet drugs after a heart attack or angina episode will be asked to take part. We hope to study 200 patients in total from throughout Glasgow.

Do I Have to Take Part?

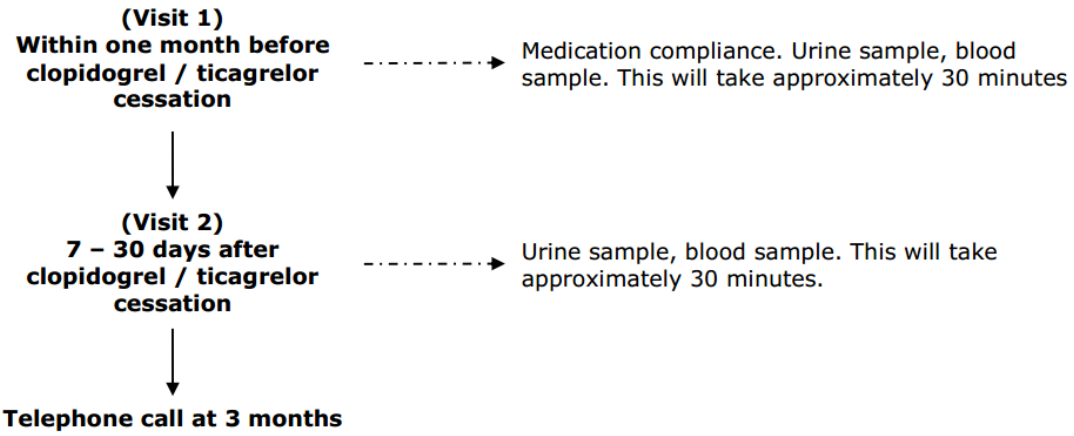
No. It is up to you to decide whether or not to take part. We will describe the study to you and go through this information sheet, which we will then give to you. If you decide to take part we will ask you to sign a consent form to show you have agreed. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What Will Happen To Me If I Take Part?

The study will involve two blood and urine samples being taken and a telephone call. Once you have agreed to take part we would clarify the date when you are due to switch to taking only one anti-platelet drug. We would then perform a blood sample and obtain a urine sample within the month before this date and repeat the samples one month after the date. We will also ask you some general questions about your tablets and each of these two visits will take about 30 minutes.

We will also telephone you to ask some questions about your health 3 months later and this should take about 10 minutes.

Taxis will be provided for visits if required and study visits will all take place in the investigations ward in the Stroke Unit of the Western Infirmary.



We will also look at your medical notes to note down the results of any relevant tests you have had to evaluate your heart attack.

What Do I Have To Do?

Other than attend the above visits, this study has no other requirements. You should continue to take all your regular medication and this study does not interfere with this.

What Are The Possible Benefits of Taking Part?

You may not derive any direct benefit from the study.

What Are The Possible Risks of Taking Part?

Sometimes people feel blood samples are uncomfortable and they are sometimes associated with mild bruising. There are no other risks to taking part.

What Happens When The Study Stops?

When the study stops you should continue all your prescribed medication and you will continue to be looked after by your cardiologist or General Practitioner.

What If There is a Problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will My Participation in the Study Be Kept Confidential?

Yes. We will follow normal ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2. We will also, with your consent, notify your General Practitioner of your participation.

This completes part 1 of this information sheet. If this has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What Happens if New Information Becomes Available?

Sometimes during the course of a research project, new information becomes available about the subject that is being studied. This is unlikely in this case but if it did happen and it affected our research plans we would tell you and discuss with you whether you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

What Will Happen If I Withdraw From The Study?

You can withdraw from study at any point and this will not affect the care you receive. If you decide to do so, we would ask if we could still use the blood and urine samples we have already taken in the study. You can refuse this and the samples can be destroyed if you wish.

What Will Happen If I lose the capacity to consent during the study?

We would not obtain further samples. However, we would ask if we could still use the blood and urine samples we have already taken in the study.

What If Something Goes Wrong?

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against NHS Greater Glasgow and Clyde but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate). There are no special compensation arrangements for non-negligent harm.

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (using the numbers below). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. In order to do this you should contact 0141 2014500 by telephone or email complaints@ggc.scot.nhs.uk.

Will My Participation In The Study Be Kept Confidential?

Yes. All data gathered during the study will be coded by a unique identifier meaning that all your personal details are removed. We will record your participation in your case notes so that other doctors involved in your care are aware. During each study visit we will keep an anonymous record on a secure University of Glasgow computer server. The study team, with Dr J Dawson as a chief investigator will act as custodians of the data and we take data protection issues very seriously. We will keep study data in a secure fashion for 10 years after which time it will be destroyed.

Your researchers will have access to study data and we will allow authorised persons from the study sponsor so they can audit quality of the research.

What Will Happen To The Samples I Give?

All blood and urine samples will be coded and identified by your unique study number. Samples will then be stored in a secure fashion in a university freezer in the Western Infirmary. Access to samples will be restricted to the researchers and the scientists who will analyse the samples. We

also seek your permission to perform further studies on these samples (in a strictly anonymous fashion) in the future for studies designed to improve care for those with heart attack. Any further studies using these samples would only take place after further review by a Research Ethics Committee.

Once we have prepared the samples we will perform what we call metabolomic analysis. This is a detailed assessment of the levels of hundreds of small molecules in the blood and we will see whether levels change after changing the drugs and if these changes differ in patients who run into further problems.

What Will Happen To The Results Of The Study?

We hope the whole study will be completed in one to two years and be published in a medical journal thereafter. There will be a possibility the results will be published at scientific meetings or conferences. We can inform you of the results should you so wish. At the same time, the results of the study will form part of Ms Mazlan-Kepli's PhD thesis.

Who Is Organising and Funding The Study?

The investigators will receive no remuneration for including you in the study. We will use Ms Mazlan-Kepli's PhD bench fees for this study. She is sponsored by Ministry of Health, Government of Malaysia Scholarship.

Who Has Reviewed the Research?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the West of Scotland Research Ethics Services.

Contact Information

If you wish any further information about the study, please contact Ms Mazlan-Kepli directly (**Tel :- 074 4811 4659**) or Dr J Dawson, via the switchboard at the Western Infirmary (**Tel :- 0141 211 2000**) or via the Acute Stroke Unit directly (**Tel :- 0141 211 2429**).

If you wish to speak to an independent person who is not involved in the study then you can contact Dr Marie Freel via the switchboard at the Western Infirmary **(**Tel :- 0141 211 2000**)**.

SUMMARY

In summary, should you agree to participate you will be asked to provide 2 blood and urine samples. The two visits will last approximately 30 minutes. We will telephone you 3 months later and look at your medical records to check you have been well. If you do not wish to participate in the study, or wish to withdraw at any time, your care will in no way be affected.

A16. Consent form

Patient Identification Number for this study:

THIS FORM HAS BEEN APPROVED BY THE WEST SCOTLAND RESEARCH ETHICS SERVICES

FORM OF CONSENT FOR PARTICIPANTS IN A CLINICAL RESEARCH PROJECT

Title of Project:

Stopping dual anti-platelet treatment following acute coronary syndrome; A metabolic analysis

Name of Researchers:

Ms W Mazlan-Kepli, Phd Student
Dr J Dawson, Clinical Senior Lecturer in Medicine
Prof M Walters, Professor of Clinical Pharmacology

Please read information below and initial boxes

1. I confirm that I have read and understand the information sheet dated 12/06/2014 (version 1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the study team or from NHS Greater Glasgow and Clyde, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree for the study team to contact me via a telephone and review my medical notes at 3 months since my last visit. ☐

5. I agree for the study team to notify my General Practitioner of my participation in the above study. ☐

6. I agree to take part in the above study. ☐

OPTIONAL

1. I agree to my anonymous samples of blood and urine being stored and used in future research projects. ☐
2. I wish to know the findings of this study via a letter. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

When completed, 1 for patient (copy); 1 for researcher site file (original); 1 in medical notes (copy).

A17. Case report form

Patient ID Number: DAPT_____

University of Glasgow

NHS Greater Glasgow and Clyde

VISIT 1 (Date:.....)

Please use (√) to indicate 'yes' in the blue columns and fill the other columns accordingly. For laboratory data, please indicate 'not available' for non-retrievable data or 'not taken' for laboratory test that is not done.

Hospital		Ward	

DEMOGRAPHICS

Gender	Male	
	Female	
Age (years)		

Ethnic Group		
Smoking Status	Never Smoked	
	Former Smoker	
	Current Smoker	
Weight		

ACUTE CORONARY SYNDROME

Types	USA	
	NSTEMI	
	STEMI	
Date of admission (dd/mm/yyyy)		
Date of discharge (dd/mm/yyyy)		
Date of death (dd/mm/yyyy)		

CONCURRENT DISEASES

Concurrent diseases	Diabetes Mellitus	
	Hypertension	
	Peripheral Vascular Disease	
	Stroke/ TIA	
	Atrial Fibrillation	
	DVT/ PE	
	Heart Failure	
	COPD/ Asthma	
	Renal Disease	
	Hyperlipidemia	
	PVD	
Others		
Family History		

ANTIPLATELET THERAPY

Indication	STEMI	
	NSTEMI	
	USA	
	DVT	
	PE	
	DVT Prophylaxis	
	Ischaemic Stroke	
Others		

ANTIPLATELET AND CONCURRENT DRUGS

DRUG	DOSE (MG)	FREQ	DATE START	DATE STOP
Aspirin				
Clopidogrel				
Ticagrelor				
Others:				

Stopping DAPT after ACS CRF Version 3
01/09/2014

Patient ID Number: DAPT_____

University of Glasgow

NHS Greater Glasgow and Clyde

COMPLIANCE – ANTIPLATELET THERAPY

Morisky Scale Score	

LABORATORY INVESTIGATIONS

Parameters/ Biomarkers		Date taken
Left ventricular ejection fraction (%)		

Sample

Sampling	Yes / No	Date taken
Blood sample		
Urine sampling		

Metabolomic measurement

Sample	Date analysis	Results
Blood		
Urine		

VISIT 2 (Date:.....)

Sample

Sampling	Yes / No	Date taken
Blood sample		
Urine sampling		

Metabolomic measurement

Sample	Analysis date	Results
Blood		
Urine		

ANTIPLATELET AND CONCURRENT DRUGS

DRUG	DOSE (MG)	FREQ	DATE START	DATE STOP
Aspirin				
Clopidogrel				
Ticagrelor				
Others:				

Patient ID Number: DAPT_____

University of Glasgow

NHS Greater Glasgow and Clyde

Day 90 (Date:.....)

Cardiovascular event

Events	ICD-10 codes	Diseases	Yes / No	Event date
ACS	I20.0	Unstable angina		
	I21	Acute myocardial infarction		
	I21.0	Acute transmural myocardial infarction of anterior wall		
	I21.1	Acute transmural myocardial infarction of inferior wall		
	I21.2	Acute transmural myocardial infarction of other sites		
	I21.3	Acute transmural myocardial infarction of unspecified site		
	I21.4	Acute subendocardial myocardial infarction		
	I21.9	Acute myocardial infarction, unspecified		
	I22	Subsequent myocardial infarction		
	I22.0	Subsequent myocardial infarction of anterior wall		
	I22.1	Subsequent myocardial infarction of inferior wall		
	I22.8	Subsequent myocardial infarction of other sites		
	I22.9	Subsequent myocardial infarction of unspecified site		
Stroke	I63	Cerebral infarction		
	I63.0	Cerebral infarction due to thrombosis of precerebral arteries		
	I63.1	Cerebral infarction due to embolism of precerebral arteries		
	I63.2	Cerebral infarction due to unspecified occlusion or stenosis of precerebral arteries		
	I63.3	Cerebral infarction due to thrombosis of cerebral arteries		
	I63.4	Cerebral infarction due to embolism of cerebral arteries		
	I63.5	Cerebral infarction due to unspecified occlusion or stenosis of cerebral arteries		
	I63.6	Cerebral infarction due to cerebral venous thrombosis, nonpyogenic		
	I63.8	Other cerebral infarction		
	I63.9	Cerebral infarction, unspecified		
Cardiac death	I46.1	Sudden cardiac death		

A18. Morisky scale

Patient Identification Number for this study: DAPT _____

**Stopping dual anti-platelet treatment following acute coronary syndrome;
A metabolic analysis**

Evaluation of Medication Compliance (Morisky Scale)

The purpose of this short questionnaire is to assess your medication compliance on the blood thinning medication which were prescribed by your cardiologist or General Practitioner.

Please circle / underline your answer.

Questions	Answers	
Do you ever forget to take your medicine?	Yes	No
Are you careless at times about taking your medicine?	Yes	No
When you feel better do you sometimes stop taking your medicine?	Yes	No
Sometimes if you feel worse when you take the medicine, do you stop taking it?	Yes	No
Total score		

Thank you for your time and participation.

A19. Metabolites detected

Sort	Trend Sort	Import Peaks	Search	Tools	Graphs	Export	confidence										
Mass	RT	FORMULA	Isomer	Putative metabolite			Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
267.1	9.472	C10H13N5O	3	Adenosine	10		Nucleotide Metabolism	C00212	11263	2.60	1.00	0.028	1	5305.835	2042.859	3569.317	1216.488
281.07	7.346	C16H11NO4	1	7-(Acetyloxy)-3-(3-pyridinyl)-2H-1-	7		0	C15048	21339	1.71	1.00	0.504	1	4332.544	2531.622	8071.799	3315.15
312.15	4.247	C18H20N2O	1	Phe-Phe	7		Peptide(di-)	0	8267	1.67	1.00	0.056	1	4650.365	2778.604	2592.921	1580.804
151.06	10.68	C8H9NO2	17	N-Methylanthranilate	5		Biosynthesis of Secondary	C03005	19072	1.63	1.00	0.490	1	4928.833	3023.821	7885.803	3293.191
240.02	13.69	C6H12N2O4	2	L-Cystine	10		Amino Acid Metabolism	C00491	5217	1.43	1.00	0.085	1	2971.26	2070.815	1859.12	2004.241
131.07	13.3	C4H9N3O2	2	Creatine	8		Amino Acid Metabolism	C00300	2109441	1.42	1.00	0.107	1	1032947	727052.9	573578.2	297620.3
297.05	12.89	C8H15N3O5	1	L-Cysteinylglycinedisulfide	7		Peptide	0	2639	1.38	1.00	0.170	1	1449.58	1047.721	917.6919	877.3961
151.06	6.371	C8H9NO2	17	(Z)-4-Hydroxyphenylacetaldehy	8		Amino Acid Metabolism	C04353	35194	1.38	1.00	0.589	1	7122.833	5157.039	12577.41	5830.475
131.06	13.07	C5H9NO3	14	L-Glutamate 5-semialdehyde	6		Amino Acid Metabolism	C01165	21662	1.33	1.00	0.278	1	14932.38	11260.45	6709.259	3155.682
187.1	13.01	C7H13N3O3	1	5-guanidino-3-methyl-2-oxo-pentanoate	7		0	0	10718	1.31	1.00	0.585	1	2411.242	1836.803	3691.866	1285.958
731.55	3.968	C40H78NO8	14	[PC (14:0/18:1)] 1-tetradecanoyl-2-(11Z-	5		Lipids: Glycerophospholipid	C00157	160946	1.28	1.00	0.229	1	94862.73	74219.25	49708.97	31598.78
230.16	4.96	C11H22N2O	2	Leu-Val	7		Peptide(di-)	0	4053	1.26	1.00	0.504	1	2191.989	1740.363	1297.418	870.27
152.05	5.067	C8H8O3	25	3,4-Dihydroxyphenylacetaldeh	8		Amino Acid Metabolism	C04043	29454	1.23	1.00	0.242	1	6098.187	4943.685	10549.42	8215.164
75.068	10.98	C3H9NO	6	(R)-1-Aminopropan-2-ol	6		Amino Acid Metabolism	C03194	473334	1.22	1.00	0.653	1	227248.6	186976.5	105352.7	155963
278.15	3.978	C16H22O4	6	2-Ethylhexyl phthalate	5		0	C03343	14056	1.20	1.00	0.297	1	9984.135	8300.093	2222.004	2412.831
773.59	3.907	C43H84NO8	20	[PE (16:0/22:1)] 1-hexadecanoyl-2-(13Z-	5		Lipids: Glycerophospholipid	C00350	25798	1.20	1.00	0.200	1	17967.8	15027.38	4922.158	4522.543
387.33	3.851	C56H86O	1	2-decaprenylphenol	7		Metabolism of Cofactors and	0	17769	1.18	1.00	0.529	1	10824.27	9189.008	3319.848	4290.715
705.53	3.985	C38H76NO8	22	[PC (15:0/15:0)] 1,2-dipentadecanoyl-sn-	5		Lipids: Glycerophospholipid	C00157	28719	1.17	1.00	0.451	1	14991.03	12863.69	8144.098	6770.685

Sort	Trend Sort	Import Peaks	Search	Tools	Graphs	Export												
Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
189.11	13.41	C7H15N3O3	1	L-Homocitrulline	7	0	C02427	6274	1.13	1.00	0.652	1	1703.038	1502.476	2111.872	1094.499
205.1	10.69	C8H15NO5	3	N-Acetyl-D-fucosamine	7	0	C15480	57250	1.13	1.00	0.432	1	11313.79	10030.91	20567.19	17850.86
115.06	12.13	C5H9NO2	4	L-Proline	10	Amino Acid Metabolism	C00148	7712238	1.12	1.00	0.644	1	4559494	4060072	2191967	1417780
129.08	10.99	C6H11NO2	9	L-Pipecolate	8	Amino Acid Metabolism	C00408	771687	1.12	1.00	0.602	1	237037.6	212208.5	251229	228476.7
743.55	3.915	C41H78NO8	18	[PE (18:0/18:2)] 1-octadecanoyl-2-(9Z,12Z-	5	Lipids: Glycerophospholipid	C00350	27537	1.11	1.00	0.382	1	16805.39	15119.97	5369.478	4508.414
327.1	10.65	C14H17NO8	1	Acetaminophenglucuronide	7	0	0	252101	1.11	1.00	0.864	1	64197.38	57942.36	108374.1	74773.88
759.58	3.918	C42H82NO8	20	[PC (16:0/18:1)] 1-hexadecanoyl-2-(11Z-	5	Lipids: Glycerophospholipid	C00157	1785396	1.11	1.00	0.442	1	1277193	1154107	330455.3	285262.9
147.05	12.72	C5H9NO4	14	L-Glutamate	10	Amino Acid Metabolism	C00025	47540	1.10	1.00	0.630	1	22846.77	20738.27	13626.97	5706.524
755.55	3.954	C42H78NO8	17	[PC (16:0/18:3)] 1-hexadecanoyl-2-	5	Lipids: Glycerophospholipid	C00157	108353	1.10	1.00	0.615	1	69334.59	63254.16	30911.43	16932.51
102.07	7.489	C5H10O2	5	Pentanoate	5	Lipids: Fatty Acyls	C00803	66927	1.09	1.00	0.563	1	37196.98	34164.36	6027.843	16886.58
125.01	13.28	C2H7NO3S	1	Taurine	8	Lipid Metabolism	C00245	65601	1.09	1.00	0.776	1	32249.44	29709.9	14609.5	14207.68
160.12	12.15	C7H16N2O2	6	Bethanechol	5	0	C06850	3578	1.08	1.00	0.680	1	2187.293	2017.977	880.7431	516.1354
733.56	3.947	C40H80NO8	24	[PC (16:0/16:0)] 1-hexadecanoyl-2-	5	Lipids: Glycerophospholipid	C00157	62267	1.08	1.00	0.585	1	43069.71	39903.13	9584.106	11770.65
767.55	3.817	C43H78NO8	31	[PE (18:0/20:4)] 1-octadecanoyl-2-	5	Lipids: Glycerophospholipid	C00350	19987	1.08	1.00	0.525	1	14991.1	13917.79	5140.431	3016.099
751.55	3.774	C43H78NO7	16	[PE (18:1/20:4)] 1-(1Z-octadecenyl)-2-	5	Lipids: Glycerophospholipid	C00350	24104	1.07	1.00	0.671	1	12627.01	11792.74	5609.368	3236.11
749.54	3.782	C43H76NO7	14	PE(20:4(5Z,8Z,11Z,14Z)/P-18:1(11Z))	5	Lipids: Glycerophospholipid	C00350	21291	1.06	1.00	0.753	1	12411.72	11658.44	5132.445	3845.458
826.69	4.006	C48H95N2O	1	SM(d19:1/24:1(15Z))	5	Lipids: Sphingolipids	C00550	6900	1.06	1.00	0.646	1	5285.173	4977.262	1505.646	991.2263
118.06	7.5	C5H10O3	12	5-Hydroxypentanoate	5	Lipids: Fatty Acyls	C02804	106858	1.06	1.00	0.805	1	51780.78	48943.41	21111.26	28154.98

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Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
116.08	4.85	C6H12O2	16	Hexanoic acid	7	Lipids: Fatty Acyls	C01585	428170	1.06	1.00	0.689	1	260819.9	247183.4	21273.04	100553.6
146.11	19.8	C6H14N2O2	8	D-Lysine	8	Amino Acid Metabolism	C00739	47227	1.05	1.00	0.841	1	28898.97	27525.01	11311.51	11535.36
152.03	10.97	C5H4N4O2	3	Xanthine	10	Nucleotide Metabolism	C00385	6817	1.05	1.00	0.868	1	3413.314	3253.071	1782.692	1351.925
501.29	4.543	C25H44NO7	4	[PE (20:4)] 1-(5Z,8Z,11Z,14Z-	5	Lipids: Glycerophospholipid	0	14294	1.04	1.00	0.771	1	10223.54	9798.836	4404.411	2851.047
276.24	3.51	C34H64O5	2	DG(15:0/16:1(9Z)/0:0)	7	Lipids: Glycerolipids	0	6393	1.04	1.00	0.797	1	2734.997	2621.949	2138.198	1299.472
783.58	3.915	C44H82NO8	21	PC(18:2(9Z,12Z)/18:1(9Z))	5	Lipids: Glycerophospholipid	C00157	906430	1.04	1.00	0.770	1	603081.6	581798.5	169314.9	165796.6
223.11	12.32	C8H17NO6	1	N-acetyl -D- glucosaminitol	5	0	0	15524	1.04	1.00	0.749	1	10498.36	10135.21	3988.836	2678.981
635.62	3.767	C41H81NO3	1	Cer(d18:1/23:0)	5	Lipids: Sphingolipids	0	10847	1.04	1.00	0.847	1	5915.641	5714.729	2540.335	2028.675
144.12	4.468	C8H16O2	11	[FA (8:0)] octanoic acid	7	Lipids: Fatty Acyls	C06423	545976	1.03	1.00	0.784	1	346869	335252.7	71889.96	132912.4
188.12	12.05	C8H16N2O3	7	N6-Acetyl-L-lysine	6	Amino Acid Metabolism	C02727	2209	1.03	1.00	0.865	1	1490.084	1444.714	561.9787	467.5577
800.68	4.062	C46H93N2O	2	SM(d18:1/23:0)	5	Lipids: Sphingolipids	C00550	18923	1.03	1.00	0.740	1	13140.26	12747.21	3364.827	2433.902
245.16	8.414	C12H23NO4	4	N-(octanoyl)-L-homoserine	7	0	0	15148	1.02	1.00	0.898	1	6538.292	6390.091	4214.547	2957.032
259.18	7.546	C13H25NO4	2	[FA (6:0)] O-hexanoyl-R-carnitine	7	Lipids: Fatty Acyls	0	19581	1.02	1.00	0.857	1	6384.731	6247.318	6083.858	5496.375
811.61	3.885	C46H86NO8	23	PC(16:1(9Z)/22:2(13Z,16Z))	5	Lipids: Glycerophospholipid	C00157	222125	1.02	1.00	0.906	1	147170.9	144410.5	55328.01	45238.99
159.09	11.75	C7H13NO3	10	5-Acetamidopentanoate	8	Amino Acid Metabolism	C03087	357720	1.01	1.00	0.973	1	72157.98	71097.49	87723.84	128512.9
174.11	21.28	C6H14N4O2	2	L-Arginine	10	Amino Acid Metabolism	C00062	91521	1.01	1.00	0.955	1	50472.81	49802.52	24223.62	14681.65
723.52	3.812	C41H74NO7	14	PE(18:3(6Z,9Z,12Z)/P-18:1(11Z))	5	Lipids: Glycerophospholipid	C00350	17966	1.01	1.00	0.901	1	8028.181	7929.837	4641.859	3762.073
467.3	4.885	C22H46NO7	3	[PC (14:0)] 1-tetradecanoyl-sn-glycero-3-	7	Lipids: Glycerophospholipid	C04230	37748	1.01	1.00	0.975	1	19198.93	19042.65	13557.05	6303.664

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Mass	RT	FORMULA	Isomer	Putative metabolite														
147.09	10.63	C6H13NO3	2	N-hydroxyisoleucine	5	0	0	14498	1.01	1.00	0.952	1	5377.828	5334.982	4563.006	4371.138
222.09	4.109	C12H14O4	8	Apiole	7	0	C10429	152559	1.01	1.00	0.971	1	98044.07	97476.12	20886.8	32462.82
427.37	4.421	C25H49NO4	1	Stearoylcarnitine	7	0	0	3625	1.00	1.00	0.996	1	2235.135	2237.628	903.1279	833.5866
649.64	3.773	C42H83NO3	3	[SP (24:0)] N-(tetracosanoyl)-sphing-4-	7	Lipids: Sphingolipids	C00195	25241	1.00	1.00	0.980	1	14077.65	14120.48	5350.343	4469.511
188.15	18.11	C9H20N2O2	2	N6,N6,N6-Trimethyl-L-lysine	6	Amino Acid Metabolism	C03793	4588	1.00	1.00	0.982	1	3307.268	3319.13	995.7354	473.959
298.25	3.569	C18H34O3	46	2-Oxooctadecanoic acid	7	Lipids: Fatty Acyls	C00869	97995	1.00	1.00	0.984	1	40418.72	40577.78	20116.76	29011.77
523.37	4.546	C26H54NO7	9	[PC (18:0)] 1-octadecanoyl-sn-glycero-3-	7	Lipids: Glycerophospholipid	0	495339	1.00	1.00	0.988	1	279464.1	280720.1	140976.4	128279.1
717.57	3.924	C40H80NO7	6	PC(14:0/P-18:0)	5	Lipids: Glycerophospholipid	C00157	14701	0.99	1.00	0.972	1	8422.056	8478.419	2989.632	2066.376
145.07	9.291	C6H11NO3	9	4-Acetamidobutanoate	8	Amino Acid Metabolism	C02946	8169	0.99	1.00	0.959	1	2647.991	2668.62	2566.123	1899.296
136.12	4.105	C10H16	30	[PR] (-)-Limonene	5	Lipids: Prenols	C00521	5098	0.99	1.00	0.936	1	3935.535	3969.721	822.6463	1094.821
786.66	4.078	C45H91N2O	2	SM(d18:1/22:0)	5	Lipids: Sphingolipids	C00550	48437	0.99	1.00	0.911	1	30830.13	31103.79	6965.437	5152.234
198.08	10.58	C7H10N4O3	1	5-Acetylamino-6-amino-3-methyluracil	7	Biosynthesis of Secondary	C16366	10607	0.99	1.00	0.978	1	2668.926	2707.486	3642.153	1903.569
71.074	11.97	C4H9N	2	3-Buten-1-amine	7	0	C12244	32374	0.98	1.00	0.905	1	22272.01	22676.43	6652.889	5696.731
88.053	9.124	C4H8O2	7	Butanoic acid	8	Carbohydrate Metabolism	C00246	22051	0.98	1.00	0.831	1	16736.91	17080.18	1808.861	2719.382
771.58	3.913	C43H82NO8	24	[PE (18:0/20:2)] 1-octadecanoyl-2-(11Z,14Z-	5	Lipids: Glycerophospholipid	C00350	61411	0.98	1.00	0.879	1	37134.48	37990.39	12233.18	13921.05
812.68	4.034	C47H93N2O	1	SM(d18:1/24:1(15Z))	5	Lipids: Sphingolipids	C00550	135981	0.98	1.00	0.824	1	90405.95	92523.42	10959.51	17376.36
163.07	11.95	C6H13NO2S	6	S-Methyl-L-methionine	8	Amino acid Metabolism	C03172	2800	0.98	1.00	0.962	1	724.8443	743.3971	956.9255	428.4084
511.33	5.179	C31H45NO5	1	[SP] Scyphostatin A	7	Lipids: Sphingolipids	0	10649	0.97	1.00	0.929	1	5743.136	5899.8	2206.865	2932.896

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Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
729.53	3.969	C40H76NO8	14	[PC (14:0/18:2)] 1-tetradecanoyl-2-(9Z,12Z-	5	Lipids: Glycerophospholipid	C00157	31043	0.97	1.00	0.869	1	16749.86	17260.73	9429.559	6250.475
495.33	7.506	C24H50NO7	5	1-Palmitoylglycerophosphoc	7	0	C04102	1273714	0.97	1.00	0.903	1	563650.4	581526.7	176048.4	307362.6
757.56	3.939	C42H80NO8	34	[PC (16:0/18:2)] 1-hexadecanoyl-2-(9Z,12Z-	7	Lipids: Glycerophospholipid	C00157	2830266	0.97	1.00	0.800	1	1901806	1962278	481687.4	474806.5
132.09	18.61	C5H12N2O2	6	L-Ornithine	8	Amino Acid Metabolism	C00077	30875	0.97	1.00	0.883	1	16653.25	17198.87	7690.138	5423.884
103.1	18.6	C5H13NO	1	Choline	10	Amino Acid Metabolism	C00114	261598	0.97	1.00	0.833	1	164420	170099.9	63074.07	62451.18
88.052	14.28	C4H8O2	7	(R)-Acetoin	8	Carbohydrate Metabolism	C00810	18251	0.97	1.00	0.421	1	15866.5	16417.95	813.5199	1413.922
621.61	3.769	C40H79NO3	2	[SP (22:0)] N-(docosanoyl)-sphing-4-enine	5	Lipids: Sphingolipids	C00195	9840	0.96	1.00	0.782	1	5847.389	6063.583	2190.666	1935.58
248.05	11.46	C9H12O8	1	pentane-1,3,4,5-tetracarboxylate	5	0	0	4271	0.96	1.00	0.771	1	2470.938	2569.833	929.1848	837.7722
785.59	3.904	C44H84NO8	55	[PC (18:1/18:1)] 1-(9Z-octadecenoyl)-2-(9Z-	5	Lipids: Glycerophospholipid	C00157	1254149	0.96	1.00	0.746	1	959606.9	1002707	192938	301259.8
745.6	3.921	C42H84NO7	12	PC(16:0/P-18:0)	5	Lipids: Glycerophospholipid	C00157	29674	0.96	1.00	0.791	1	17565.45	18366.96	3989.624	7895.894
257.1	12.9	C8H20NO6P	1	sn-glycero-3-Phosphocholine	10	Lipid Metabolism	C00670	2916	0.96	1.00	0.879	1	1694.462	1772.059	771.4794	711.8139
212.14	4.146	C12H20O3	9	[FA oxo(12:1)] 12-oxo-10E-dodecenoic acid	5	Lipids: Fatty Acyls	C16309	2448	0.95	1.00	0.741	1	1437.109	1505.72	289.1197	478.5325
543.33	4.558	C28H50NO7	3	LysoPC(20:4(5Z,8Z,11Z,14Z))	5	Lipids: Glycerophospholipid	C04230	187463	0.95	1.00	0.799	1	94426.36	99336.09	40092.7	40139
196.06	12.37	C6H12O7	11	D-Gluconic acid	10	Carbohydrate Metabolism	C00257	8821	0.94	1.00	0.696	1	5420.822	5737.381	1999.914	1726.587
172.15	4.006	C10H20O2	9	Decanoic acid	8	Lipid Metabolism	C01571	87712	0.94	1.00	0.797	1	52728.83	55887.31	23832.26	19750.26
160.12	19.11	C7H16N2O2	6	N6-Methyl-L-lysine	8	Amino Acid Metabolism	C02728	4247	0.94	1.00	0.692	1	1557.631	1657.008	1137.747	1303.961
165.08	10.35	C9H11NO2	7	L-Phenylalanine	10	Amino Acid Metabolism	C00079	198221	0.94	1.00	0.764	1	116384.9	124413.9	53356.5	48442.35
247.97	11.68	C8H9BrO4	1	4-bromoisophthalate	5	0	0	11138	0.93	1.00	0.811	1	4983.832	5331.107	1151.374	3161.039

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Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
741.57	3.921	C42H80NO7	10	[PC (16:1/18:2)] 1-(1Z-hexadecenyl)-2-(9Z,12Z-	5	Lipids: Glycerophospholipid	0	45113	0.93	1.00	0.713	1	26297.58	28132.65	8845.752	11015.74
145.07	12.95	C6H11NO3	9	6-Amino-2-oxohexanoate	8	Amino Acid Metabolism	C03239	44689	0.93	1.00	0.931	1	6957.071	7444.69	16639.06	10883.67
175.1	13.84	C6H13N3O3	3	L-Citrulline	10	Amino Acid Metabolism	C00327	28421	0.93	1.00	0.787	1	14730.01	15795.51	6969.144	5546.569
202.13	11.93	C9H18N2O3	3	Leu-Ala	5	Peptide(di-)	0	10318	0.93	1.00	0.708	1	5044.519	5419.128	3183.302	1533.32
399.34	4.583	C23H45NO4	1	[FA] O-Palmitoyl-R-carnitine	6	Lipids: Fatty Acyls	C02990	14928	0.93	1.00	0.787	1	7257.148	7799.903	4058.136	3635.98
798.66	4.075	C46H91N2O	1	SM(d17:1/24:1(15Z))	5	Lipids: Sphingolipids	0	29153	0.93	1.00	0.386	1	20365.54	21925.29	5274.571	3708.768
132.05	13.48	C4H8N2O3	6	L-Asparagine	10	Amino Acid Metabolism	C00152	13603	0.93	1.00	0.754	1	6012.579	6479.363	2333.171	2649.911
180.06	13.21	C6H12O6	57	myo-Inositol	8	Carbohydrate Metabolism	C00137	447990	0.93	1.00	0.610	1	247561.8	267189.9	41219.57	84287.56
807.58	3.903	C46H82NO8	29	[PC (18:1/20:4)] 1-(9Z-octadecenoyl)-2-	5	Lipids: Glycerophospholipid	C00157	475852	0.92	1.00	0.681	1	301514.4	328632	76632.51	111979.9
821.63	3.884	C48H88NO7	6	PC(22:4(7Z,10Z,13Z,16Z)/P-18:0)	5	Lipids: Glycerophospholipid	C00157	13217	0.91	1.00	0.647	1	8034.449	8835.83	1874.091	3765.101
784.65	4.082	C45H89N2O	2	SM(d18:1/22:1(13Z))	5	Lipids: Sphingolipids	C00550	73360	0.91	1.00	0.401	1	50179.57	55222.97	14741.19	11337.75
143.09	10.67	C7H13NO2	2	Stachydrine	7	0	C10172	16459763	0.91	1.00	0.523	1	2535518	2794589	5204174	6052255
202.14	18.1	C8H18N4O2	3	NG,NG-Dimethyl-L-arginine	7	0	C03626	10119	0.91	1.00	0.622	1	7060.506	7794.401	2630.252	1777.243
149.05	11.24	C5H11NO2S	5	L-Methionine	10	Amino Acid Metabolism	C00073	178458	0.90	1.00	0.781	1	81113.18	89633.9	48814.99	54717.3
119.06	13.07	C4H9NO3	11	L-Threonine	8	Amino Acid Metabolism	C00188	72901	0.90	1.00	0.661	1	44892.28	49609.85	15750.88	17612.73
163.08	11.06	C6H13NO4	9	1-deoxynojirimycin	5	0	C16843	2192	0.90	1.00	0.499	1	1101.539	1219.311	536.0768	659.3806
162.05	13.07	C6H10O5	25	2-Dehydro-3-deoxy-L-rhamnonate	8	Carbohydrate Metabolism	C03979	143804	0.90	1.00	0.547	1	81118.6	89867.71	18831.49	28845.67
835.61	3.877	C48H86NO8	25	[PC (18:0/22:5)] 1-octadecanoyl-2-	5	Lipids: Glycerophospholipid	C00157	69022	0.90	1.00	0.606	1	38010.88	42208.21	7647.536	16127.05

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Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
809.59	3.901	C46H84NO8	24	[PC (18:1/20:3)] 1-(9Z-octadecenoyl)-2-	5	Lipids: Glycerophospholipid	C00157	1243816	0.90	1.00	0.566	1	572899.6	639001.9	216245	298272.8
819.61	3.884	C48H86NO7	10	PC(22:4(7Z,10Z,13Z,16Z)/P-18:1(11Z))	5	Lipids: Glycerophospholipid	C00157	19106	0.90	1.00	0.538	1	10113.7	11282.38	2993.178	4730.599
120.05	10.89	C3H8N2O3	1	serine hydroxamate	5	0	0	3558	0.89	1.00	0.350	1	2181.947	2439.832	901.0062	1099.409
829.56	3.926	C48H80NO8	23	[PC (18:2/22:6)] 1-(9Z,12Z-octadecadienoyl)-2-	5	Lipids: Glycerophospholipid	C00157	9695	0.89	1.00	0.524	1	5873.262	6577.518	1207.6	2276.431
119.06	10.65	C4H9NO3	11	L-Allothreonine	8	Amino Acid Metabolism	C05519	24530	0.89	1.00	0.734	1	6705.43	7509.632	3995.565	7738.766
109.05	10.02	C6H7NO	7	2-Aminophenol	8	Amino Acid Metabolism	C01987	19279	0.89	1.00	0.752	1	5211.205	5855.821	6924.361	6183.824
702.57	4.233	C39H79N2O	1	[SP (16:0)] N-(hexadecanoyl)-sphing-4-	7	Lipids: Sphingolipids	0	562912	0.89	1.00	0.227	1	400947.5	450816.1	60267.61	81856.18
246.09	10.2	C9H14N2O6	2	5-6-Dihydrouridine	5	0	0	4623	0.89	1.00	0.579	1	2464.773	2773.071	1013.794	856.9914
795.58	3.911	C45H82NO8	22	PE(18:0/22:4(7Z,10Z,13Z,16Z))	5	Lipids: Glycerophospholipid	C00350	40400	0.89	1.00	0.537	1	20481.11	23085.19	6321.921	8908.435
758.63	4.162	C43H87N2O	1	SM(d18:1/20:0)	5	Lipids: Sphingolipids	C00550	38033	0.89	1.00	0.324	1	25736.9	29035.1	10161.96	6135.621
647.62	3.761	C42H81NO3	1	[SP (24:0)] N-(15Z-tetracosenoyl)-sphing-4-	5	Lipids: Sphingolipids	C00195	18621	0.89	1.00	0.476	1	11382.18	12856.34	4253.058	4163.689
97.968	14.45	H2O4S	1	Sulfate	8	Nucleotide Metabolism	C00059	106191	0.88	1.00	0.276	1	66743.35	75501.52	19925.26	17241.77
795.61	3.895	C46H86NO7	12	PC(20:2(11Z,14Z)/P-18:1(11Z))	5	Lipids: Glycerophospholipid	C00157	35153	0.88	1.00	0.469	1	22140.49	25074.23	5905.632	8825.724
156.05	10.6	C6H8N2O3	4	4-Imidazolone-5-propanoate	6	Amino Acid Metabolism	C03680	19300	0.88	1.00	0.648	1	6788.192	7691.795	2199.768	5285.012
743.58	3.937	C42H82NO7	14	1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-	5	Lipids: Glycerophospholipid	C13876	42519	0.88	1.00	0.481	1	22590.93	25608.96	6484.574	9682.203
150.05	13.22	C5H10O5	37	D-Xylulose	6	Carbohydrate Metabolism	C00310	14624	0.88	1.00	0.532	1	7143.337	8103.189	1937.442	3172.274
239.09	9.043	C15H13NO2	5	N-Hydroxy-2-acetamidofluorene	5	0	C03954	3101	0.88	1.00	0.450	1	1742.498	1976.691	836.6667	628.5898
136.04	10.35	C5H4N4O	3	**Hypoxanthine**	10	Nucleotide Metabolism	C00262	66354	0.88	1.00	0.793	1	17096.69	19452.42	8905.297	20827.61

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Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
89.048	13.31	C3H7NO2	9	L-Alanine	8	Amino Acid Metabolism	C00041	389624	0.88	1.00	0.636	1	195141.4	222142	61459.18	109088.5
120.04	13.33	C4H8O4	9	D-Erythrose	6	Carbohydrate Metabolism	C01796	92477	0.88	1.00	0.461	1	44028.17	50143.99	9073.273	19818.52
102.03	13.46	C4H6O3	8	2-Oxobutanoate	6	Amino Acid Metabolism	C00109	212646	0.88	1.00	0.504	1	119146	135810.8	33466.53	46555.09
730.6	4.195	C41H83N2O	3	SM(d18:0/18:1(9Z))	5	Lipids: Sphingolipids	C00550	94862	0.88	1.00	0.326	1	49119.4	56030.42	14670.63	18758.89
90.032	13.4	C3H6O3	6	3-Hydroxypropanoate	8	Amino Acid Metabolism	C01013	353366	0.87	1.00	0.549	1	172102.9	196768.3	36872.49	79971.2
688.55	4.245	C38H77N2O	1	[SP (18:0/14:0)] N-(octadecanoyl)-	5	Lipids: Sphingolipids	0	34776	0.87	1.00	0.250	1	22169.79	25369.5	7013.767	6556.133
88.016	13.19	C3H4O3	3	3-Oxopropanoate	6	Carbohydrate Metabolism	C00222	70415	0.87	1.00	0.488	1	37276.43	42668.92	8975.408	15173.66
174.02	14.18	C6H6O6	3	cis-Aconitate	10	Carbohydrate Metabolism	C00417	31434	0.87	1.00	0.703	1	9073.959	10402.83	3842.191	5777.532
135.04	11.43	C4H9NO2S	3	L-Homocysteine	6	Amino Acid Metabolism	C00155	6722	0.87	1.00	0.686	1	2765.408	3172.481	1678.244	1731.801
204.09	11.5	C11H12N2O	6	L-Tryptophan	10	Amino Acid Metabolism	C00078	143172	0.87	1.00	0.643	1	69696.58	80013.49	35414.16	39738.97
132.04	13.22	C5H8O4	16	2-Acetolactate	6	Carbohydrate Metabolism	C00900	58939	0.87	1.00	0.512	1	31362.16	36046.04	9594.063	13767.97
213.01	7.458	C8H7NO4S	1	Indoxylsulfate	7	0	0	1058514	0.87	1.00	0.542	1	321659.1	370123.2	198718	318447.8
674.54	4.272	C37H75N2O	1	SM(d18:1/14:0)	5	Lipids: Sphingolipids	0	101391	0.87	1.00	0.223	1	56649.38	65377.51	21528.94	19438.66
90.032	12.09	C3H6O3	6	Glyceraldehyde	8	Lipid Metabolism	C02154	590338	0.87	1.00	0.598	1	279482.7	322806.9	75565	153825.6
271.04	11.68	C9H9N3O7	2	3,5-Dinitro-L-tyrosine	5	0	C03225	5042	0.86	1.00	0.667	1	1873.133	2165.81	527.3038	1436.096
146.07	13.34	C5H10N2O3	6	L-Glutamine	8	Amino Acid Metabolism	C00064	1238784	0.86	1.00	0.507	1	647974.6	751593.1	188513.6	262678.1
114.03	13.27	C5H6O3	6	2-Hydroxy-2,4-pentadienoate	6	Amino Acid Metabolism	C00596	126581	0.86	1.00	0.517	1	64582.71	74936.47	20599.58	30156.45
769.6	3.909	C44H84NO7	15	PC(18:1(11Z)/P-18:1(11Z))	5	Lipids: Glycerophospholipid	C00157	31857	0.86	1.00	0.386	1	15237.72	17703.63	4042.048	7894.394

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Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
216.12	13.28	C8H16N4O3	2	N-acetyl-(L)-arginine	5	0	0	8722	0.86	1.00	0.499	1	4265.295	4965.121	1369.878	2140.313
275.11	12.95	C10H17N3O	7	Gamma-Glutamylglutamine	5	Peptide	C05283	2840	0.86	1.00	0.627	1	1570.684	1829.254	997.6434	436.6563
231.15	9.206	C11H21NO4	3	O-Butanoylcarnitine	7	Lipids: Fatty Acyls	C02862	40598	0.86	1.00	0.264	1	16599.23	19359.34	11443.87	11091.2
181.1	10.35	C7H11N5O	1	6-methyltetrahydropterin	5	0	0	63745	0.86	1.00	0.761	1	15814.66	18454.12	7868.932	20217.89
833.59	3.877	C48H84NO8	27	[PC (18:1/22:5)] 1-(11Z-octadecenoyl)-2-	5	Lipids: Glycerophospholipid	C00157	244821	0.86	1.00	0.500	1	107505.4	125564.4	30957.56	61118.35
358.97	7	C6H5HgO3S	1	4-mercuriphenylsulfonate	5	0	0	2553	0.85	1.00	0.750	1	689.5012	807.9038	1044.442	1068.31
105.04	13.85	C3H7NO3	3	L-Serine	6	Amino Acid Metabolism	C00065	33507	0.85	1.00	0.530	1	17297.93	20298.46	5884.96	8291.912
134.06	9.883	C5H10O4	8	Deoxyribose	8	Carbohydrate Metabolism	C01801	28786	0.85	1.00	0.710	1	9568.148	11232.69	9992.718	10666.31
79.926	11.84	BrH	1	Br-	7	0	C01324	28777	0.85	1.00	0.428	1	10826.32	12759.99	3349.23	7603.908
509.35	4.631	C25H52NO7	8	LysoPC(17:0)	5	Lipids: Glycerophospholipid	C04230	35983	0.85	1.00	0.604	1	15951.3	18805.77	10341.65	9732.15
182.04	9.788	C6H6N4O3	4	1-Methyluric acid	5	Biosynthesis of Secondary	C16359	8462	0.85	1.00	0.598	1	2740.505	3238.775	2993.744	2454.399
300.27	3.732	C18H36O3	27	[FA hydroxy(18:0)] 2S-hydroxy-octadecanoic acid	5	Lipids: Fatty Acyls	C03045	6413	0.84	1.00	0.337	1	3395.178	4018.034	858.8372	1489.18
150.05	11.11	C5H10O5	37	D-Ribose	10	Carbohydrate Metabolism	C00121	48959	0.84	1.00	0.583	1	19613.85	23216.35	15616.74	17572.11
313.14	10.83	C12H19N5O	2	Ala-Ser-His	5	Peptide(tri-)	0	3054	0.84	1.00	0.213	1	1863.438	2206.843	607.1873	460.6065
166.05	12.23	C5H10O6	8	L-Arabinonate	8	Carbohydrate Metabolism	C00545	7780	0.84	1.00	0.425	1	3675.548	4358.029	2184.531	1838.459
181.07	12.31	C9H11NO3	11	3-Amino-3-(4-hydroxyphenyl)propanoat	6	Amino Acid Metabolism	C04368	214989	0.84	1.00	0.569	1	93151.62	110499.9	65430.11	55510.74
264.64	4.402	C26H43NO8	3	Glycochenodeoxycholate 7-sulfate	7	0	C15559	8365	0.84	1.00	0.257	1	3203.742	3814.246	1754.539	2511.311
160.11	4.366	C8H16O3	14	Ethyl (R)-3-hydroxyhexanoate	7	0	C03864	4014	0.84	1.00	0.275	1	2325.449	2770.684	765.6546	767.2552

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168.12	4.12	C10H16O2	60	(3S,6R)-6-Isopropenyl-3-methyl-2-oxo-oxepanone	5	Biosynthesis of Secondary	C11403	4621	0.84	1.00	0.170	1	2436.581	2903.826	1301.104	799.055
831.58	3.892	C48H82NO8	22	[PC (18:1/22:6)] 1-(11Z-octadecenoyl)-2-	5	Lipids: Glycerophospholipid	C00157	45361	0.84	1.00	0.461	1	21736.51	25926.15	5545.234	13316.06
246.14	9.103	C14H18N2O	2	Hypaphorine	7	0	C09213	142785	0.84	1.00	0.402	1	41312.62	49358.39	49388.78	41894.84
312.23	4.092	C18H32O4	39	[FA (18:2)] 9S-hydroperoxy-10E,12Z-octadecadienoic	7	Lipids: Fatty Acyls	C14827	35537	0.84	1.00	0.579	1	10978	13118.76	5039.793	10912.76
179.08	11.06	C6H13NO5	10	D-Galactosamine	8	Carbohydrate Metabolism	C02262	45136	0.84	1.00	0.391	1	22677.65	27141.4	11951.34	14846.71
164.07	11.61	C6H12O5	25	L-Rhamnulose	6	Carbohydrate Metabolism	C00861	205695	0.84	1.00	0.318	1	114901.6	137521.2	35270.54	37864.56
646.51	4.3	C35H71N2O	1	SM(d18:1/12:0)	7	Lipids: Sphingolipids	C00550	14255	0.83	1.00	0.316	1	4362.558	5235.112	4447.576	4158.814
166.06	5.017	C9H10O3	17	3-(3-Hydroxy-phenyl)-propanoic acid	8	Amino Acid Metabolism	C11457	5402	0.83	1.00	0.440	1	2439.041	2931.784	1028.633	1373.348
805.56	3.91	C46H80NO8	28	[PC (16:0/22:6)] 1-hexadecanoyl-2-	5	Lipids: Glycerophospholipid	C00157	927465	0.83	1.00	0.404	1	377181.2	454870	120099.1	226808.6
425.35	4.465	C25H47NO4	3	Elaidiccarnitine	5	0	0	19346	0.83	1.00	0.430	1	8912.625	10789.86	3650.858	4149.339
99.956	12.22	ClHO4	1	perchlorate	7	0	0	15065	**0.82**	1.00	0.010	1	10137.62	12323.04	1284.797	1799.597
147.09	11.7	C6H13NO3	2	Fagomine	7	0	C10144	18558	0.82	1.00	0.528	1	6650.383	8107.107	2319.163	5289.512
140.98	15.49	CH4NO5P	1	Carbamoyl phosphate	6	Amino Acid Metabolism	C00169	3428	0.82	1.00	0.086	1	2349.031	2866.37	705.1428	239.7841
244.07	10.04	C9H12N2O6	3	Uridine	8	Nucleotide Metabolism	C00299	26218	0.81	1.00	0.538	1	11346.07	13937.28	5844.915	7519.832
75.032	13.83	C2H5NO2	3	Glycine	8	Amino Acid Metabolism	C00037	26284	0.81	1.00	0.324	1	12001.67	14743.7	3738.131	6196.16
208.08	10.9	C10H12N2O	2	L-Kynurenine	8	Amino Acid Metabolism	C00328	7867	0.81	1.00	0.475	1	3716.394	4582.122	1719.233	1967.439
228.15	12.16	C11H20N2O	3	Ile-Pro	5	Peptide(di-)	0	35512	0.81	1.00	0.555	1	10559.45	13075.76	4967.917	10208.98
208.06	11.57	C7H12O7	1	1-O-methyl-β-D-glucuronate	7	0	0	3920	0.80	1.00	0.110	1	2536.525	3151.099	477.024	958.4695

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Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
192.06	11.58	C7H12O6	6	Quinate	8	Amino Acid Metabolism	C00296	56746	0.80	1.00	0.418	1	21197.8	26425.34	22135.53	24324.67
229.17	4.75	C12H23NO3	1	N-Decanoylglycine	7	0	0	3957	0.79	1.00	0.304	1	1856.278	2340.643	305.9249	1111.567
279.15	8.291	C15H21NO4	1	Metalaxyl	7	0	C10947	6080	0.79	1.00	0.434	1	2745.531	3463.84	1604.364	2095.43
146.02	13.09	C5H6O5	7	2-Oxoglutarate	10	Carbohydrate Metabolism	C00026	115989	0.79	1.00	0.510	1	34702.76	43888.58	13663.7	32879.14
216.15	11.84	C10H20N2O	1	Val-Val	7	Peptide(di-)	0	10330	0.79	1.00	0.498	1	2191.45	2776.695	2202.331	3556.279
217.13	9.986	C10H19NO4	1	O-Propanoylcarnitine	5	Lipids: Fatty Acyls	C03017	111926	0.78	1.00	0.316	1	48525.16	61930.61	30362.61	31562.6
268.08	10.83	C10H12N4O	3	Inosine	8	Nucleotide Metabolism	C00294	17322	0.78	1.00	0.082	1	6874.281	8776.792	3171.563	4116.435
728.58	4.168	C41H81N2O	2	SM(d18:1/18:1(9Z))	5	Lipids: Sphingolipids	C00550	81606	0.78	1.00	0.044	1	39062.98	49921.18	15280.56	19256.16
567.33	4.488	C30H50NO7	2	[PC (22:6)] 1-(4Z,7Z,10Z,13Z,16Z,19Z-	5	Lipids: Glycerophospholipid	C04230	35476	0.78	1.00	0.374	1	14130.25	18086.71	5343.362	8680.364
281.11	13.6	C11H15N5O	6	1-Methyladenosine	7	0	C02494	4755	0.78	1.00	0.338	1	1797.807	2302.718	579.8348	1122.685
423.34	4.513	C25H45NO4	2	Linoelaidylcarnitine	5	0	0	9797	0.78	1.00	0.370	1	3832.458	4919.331	1270.327	2674.323
168.03	11.56	C5H4N4O3	1	Urate	8	Nucleotide Metabolism	C00366	503691	0.78	1.00	0.172	1	221651.6	284533.5	34078.34	103364.3
147.05	10.22	C5H9NO4	14	L-4-Hydroxyglutamate semialdehyde	6	Amino Acid Metabolism	C05938	18746	0.78	1.00	0.364	1	6713.941	8646.445	2515.027	4559.026
429.37	3.769	C29H49O2	1	4alpha-hydroxymethyl-4beta-methyl-5alpha-	7	0	0	40938	0.78	1.00	0.366	1	14112.76	18193.86	4201.967	10469.29
200.18	3.866	C12H24O2	10	Dodecanoic acid	8	Lipid Metabolism	C02679	237380	0.77	1.00	0.393	1	83200.46	107639.1	68872.8	44158.7
270.22	3.628	C16H30O3	19	[FA oxo(16:0)] 3-oxo-hexadecanoic acid	5	Lipids: Fatty Acyls	0	49568	0.77	1.00	0.272	1	17199.87	22254.39	7857.359	15313.63
97.977	11.96	H3O4P	1	Orthophosphate	8	Energy Metabolism	C00009	14041413	0.77	1.00	0.283	1	5843626	7564537	1489153	3085452
196.15	3.731	C12H20O2	29	Linalyl acetate	7	Lipids: Prenols	C09863	7954	0.77	1.00	0.085	1	3501.469	4565.109	2271.314	2201.952

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189.1	10.98	C8H15NO4	5	(2S)-2-{[1-(R)-Carboxyethyl]amino}penta	5	0	C06326	5242	0.77	1.00	0.291	1	2368.314	3090.471	1035.898	1232.842
129.04	9.949	C5H7NO3	6	L-1-Pyrroline-3-hydroxy-5-carboxylate	6	Amino Acid Metabolism	C04281	122989	0.77	1.00	0.283	1	65011.24	84924.28	26376.34	21958.92
117.05	14.04	C3H7N3O2	1	Guanidinoacetate	8	Amino Acid Metabolism	C00581	15627	0.76	1.00	0.260	1	6197.552	8126.862	2504.251	2917.225
105.08	11	C4H11NO2	2	Diethanolamine	8	Lipid Metabolism	C06772	27184	0.76	1.00	0.449	1	9768.943	12816.82	7426.721	8319.898
314.25	3.911	C18H34O4	12	[FA hydroxy(18:1)] 9,10-dihydroxy-12Z-	5	Lipids: Fatty Acyls	C14828	3610	0.76	1.00	0.223	1	1970.074	2600.052	742.9468	904.0943
113.06	10	C4H7N3O	1	Creatinine	10	Amino Acid Metabolism	C00791	6588377	0.76	1.00	0.372	1	2322650	3068262	952449.1	1727942
133.04	11.24	C4H7NO4	4	Iminodiacetate	5	0	0	9974	0.75	1.00	0.307	1	2563.146	3406.77	1275.987	2948.748
240.21	3.756	C15H28O2	5	[FA dimethyl(13:0)] 2,5-dimethyl-2E-tridecenoic	5	Lipids: Fatty Acyls	0	4349	0.75	1.00	0.343	1	2074.88	2758.733	991.0176	1305.804
192.03	14.4	C6H8O7	12	Citrate	8	Carbohydrate Metabolism	C00158	98667	0.75	1.00	0.374	1	39233.75	52303.89	13329.9	30497.52
83.961	12.06	HO3Cl	1	Chlorate	5	0	C01485	25316	0.75	1.00	0.141	1	12906.4	17261.3	3219.9	4862.99
161.11	12.31	C7H15NO3	2	L-Carnitine	10	Amino Acid Metabolism	C00487	13258027	0.75	1.00	0.264	1	4689628	6273018	2451534	3581697
261.12	11.64	C11H19NO6	4	Lotaustralin	5	0	C08334	2729	0.75	1.00	0.175	1	1113.03	1489.906	467.094	620.9934
104.05	7.534	C4H8O3	13	4-Hydroxybutanoic acid	6	Carbohydrate Metabolism	C00989	462195	0.75	1.00	0.330	1	166668.1	223210.9	65995.17	138049.4
453.29	4.738	C21H44NO7	5	[PE (16:0)] 1-hexadecanoyl-sn-glycero-3-	5	Lipids: Glycerophospholipid	0	13928	0.75	1.00	0.263	1	6203.547	8310.643	3418.581	3544.84
134.02	13.33	C4H6O5	4	(S)-Malate	8	Carbohydrate Metabolism	C00149	198315	0.74	1.00	0.570	1	45203.24	60676.2	17104.21	62760.09
185.11	11.01	C9H15NO3	5	Ecgonine	5	Biosynthesis of Secondary	C10858	38350	0.74	1.00	0.202	1	17196.31	23162.56	13696.55	12676.78
136.04	11.22	C4H8O5	3	[FA trihydroxy(4:0)] 2,3,4-trihydroxy-butanoic acid	8	Carbohydrate Metabolism	C01620	169250	0.74	1.00	0.209	1	71397.45	96384.39	24287.82	36842.11
81.982	11.99	H3O3P	1	Phosphonate	5	0	C06701	57415	0.74	1.00	0.413	1	19039.42	25785.32	8636.2	15646.38

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268.2	3.655	C16H28O3	2	[FA oxo(5:2/5:0/6:0)] (1R,2R)-3-oxo-2-pentyl-	5	Lipids: Fatty Acyls	0	7231	0.74	1.00	0.066	1	3671.13	4976.633	1956.568	2186.451
296.24	3.611	C18H32O3	33	[FA hydroxy(18:2)] 9S-hydroxy-10E,12Z-	5	Lipids: Fatty Acyls	C14767	16303	0.73	1.00	0.254	1	6583.051	8958.226	3723.29	5053.96
242.22	3.755	C15H30O2	9	[FA methyl(14:0)] 12-methyl-tetradecanoic acid	5	Lipids: Fatty Acyls	C16665	60528	0.73	1.00	0.156	1	30039.8	41087.55	9214.215	16637.27
272.24	3.701	C16H32O3	18	16-hydroxypalmitate	5	0	0	24658	0.73	1.00	0.219	1	8711.584	11922.32	2205.812	6229.751
275.14	11.56	C12H21NO6	1	Glutarylcarnitine	5	0	0	4808	0.73	1.00	0.172	1	1834.159	2514.175	854.7711	1170.334
495.33	4.705	C24H50NO7	5	[PC (16:0)] 1-hexadecanoyl-sn-glycero-3-	7	Lipids: Glycerophospholipid	C04230	2166529	0.73	1.00	0.266	1	1067251	1463474	551451.1	566591.4
157.11	10.2	C8H15NO2	2	Homostachydrine	7	0	C08283	15031	0.73	1.00	0.447	1	3985.505	5492.875	4065.21	5239.751
188.14	3.957	C10H20O3	13	10-Hydroxydecanoic acid	7	Lipids: Fatty Acyls	C02774	21949	0.72	1.00	0.424	1	6837.803	9437.837	1980.565	7180.837
214.13	9.997	C10H18N2O	2	Dethiobiotin	6	Metabolism of Cofactors and	C01909	6176	0.72	1.00	0.366	1	1806.801	2503.528	961.3738	1664.497
166.1	4.154	C10H14O2	17	10-oxogeranial	7	0	C17622	32480	0.72	1.00	0.306	1	12634.63	17525.78	8732.175	10072.13
116.05	5.222	C5H8O3	9	2-Oxopentanoic acid	7	Lipids: Fatty Acyls	C06255	137073	0.71	1.00	0.265	1	46709.71	65330.66	11836.89	34575.69
242.19	3.706	C14H26O3	11	[FA oxo(14:0)] 3-oxo-tetradecanoic acid	5	Lipids: Fatty Acyls	0	7383	0.71	1.00	0.051	1	3064.362	4307.072	1621.395	1991.403
92.047	10.45	C3H8O3	1	Glycerol	8	Carbohydrate Metabolism	C00116	313010	0.71	1.00	0.186	1	117372	165163.8	18758.19	80500.21
316.26	3.583	C18H36O4	19	[FA hydroxy(18:0)] 9,10-dihydroxy-octadecanoic	5	Lipids: Fatty Acyls	0	28300	0.71	1.00	0.374	1	8157.077	11528.41	4337.39	9531.683
256.24	3.728	C16H32O2	16	Hexadecanoic acid	8	Lipid Metabolism	C00249	3447226	0.70	1.00	0.082	1	1561533	2216802	310078	930036.5
195.05	7.509	C9H9NO4	9	Dopaquinone	6	Amino Acid Metabolism	C00822	483626	0.70	1.00	0.498	1	145854.9	207965.4	146699.3	149355.7
228.15	10.23	C11H20N2O	3	Leu-Pro	7	Peptide(di-)	0	116917	0.70	1.00	0.306	1	29939.85	42729.81	10706.82	33199.53
126.02	4.654	C7H7Cl	2	4-Chlorotoluene	5	0	C14451	7354	0.70	1.00	0.186	1	2961.684	4227.689	1556.439	1914.377

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104.02	10.87	C2H4N2O3	1	Urea-1-carboxylate	8	Amino Acid Metabolism	C01010	1292117	0.70	1.00	0.313	1	306444.5	438028.7	149501.1	397912.5
145.11	12.33	C7H15NO2	6	4-Trimethylammoniobutano	8	Amino Acid Metabolism	C01181	238425	0.70	1.00	0.240	1	98252.23	141253.7	45394.46	70551.53
117.08	11.06	C5H11NO2	16	Betaine	10	Amino Acid Metabolism	C00719	14064975	0.70	1.00	0.226	1	5542104	7974214	4324789	5012910
190.05	10.34	C7H10O6	4	[FA hydroxy,oxo(7:0/2:0)] 4-hydroxy-2-oxo-	8	Amino Acid Metabolism	C05601	20840	0.69	1.00	0.299	1	4719.707	6838.922	2574.658	6320.477
131.09	11.04	C6H13NO2	12	L-Leucine	10	Amino Acid Metabolism	C00123	1009272	0.69	1.00	0.257	1	383323.8	559464.6	195295.6	328725.1
159.13	12.33	C8H17NO2	4	DL-2-Aminooctanoicacid	5	0	0	483242	0.68	1.00	0.296	1	119048.4	173804.7	85038.17	162709.3
162.02	12.21	C5H6O6	2	4-Hydroxy-2-oxoglutarate	8	Carbohydrate Metabolism	C01127	4723	0.68	1.00	0.399	1	1070.46	1563.308	1178.745	1642.88
507.33	4.681	C25H50NO7	3	[PC (17:0)] 1-(10Z-heptadecenoyl)-sn-glycero-	5	Lipids: Glycerophospholipid	0	8223	0.68	1.00	0.333	1	2637.576	3861.158	1654.124	2294.314
166.05	8.565	C6H6N4O2	3	7-Methylxanthine	7	Biosynthesis of Secondary	C16353	10369	0.68	1.00	0.258	1	3815.916	5592.373	3661.992	2809.516
254.12	4.597	C13H18O5	1	[FA methyl,hydroxy,oxo(5:2/4:	7	Lipids: Fatty Acyls	0	13967	0.68	1.00	0.522	1	2394.782	3510.948	964.8583	4849.406
397.32	4.633	C23H43NO4	2	trans-Hexadec-2-enoylcarnitine	5	0	0	5712	0.68	1.00	0.202	1	1852.675	2728.09	807.1007	1412.101
177.08	10.58	C10H11NO2	3	5-Hydroxytryptophol	5	0	0	5978	0.68	1.00	0.243	1	1569.219	2315.184	746.0098	1707.26
285.19	5.236	C15H27NO4	1	2-Octenoylcarnitine	7	0	0	45911	0.68	1.00	0.194	1	15047.17	22263.04	7200.123	13063.28
584.26	3.644	C33H36N4O	4	Bilirubin	8	Metabolism of Cofactors and	C00486	238627	0.67	1.00	0.137	1	61366.72	90943.11	47602.24	72698.85
184.15	4.014	C11H20O2	17	[FA (11:1)] 10-undecenoic acid	7	Lipids: Fatty Acyls	C13910	13099	0.67	1.00	0.041	1	6119.259	9126.558	3748.438	3245.279
212.18	3.678	C13H24O2	6	[FA methyl(12:1)] 2-methyl-2-dodecenoic acid	5	Lipids: Fatty Acyls	0	3453	0.67	1.00	0.039	1	1516.148	2265.707	588.5354	841.1156
169.09	11.96	C7H11N3O2	5	N(pi)-Methyl-L-histidine	10	Amino Acid Metabolism	C01152	94111	0.67	1.00	0.145	1	17716.62	26558.52	22947.29	32073.28
216.17	3.582	C12H24O3	11	12-Hydroxydodecanoic acid	5	Lipids: Fatty Acyls	C08317	5826	0.66	1.00	0.229	1	1545.561	2348.965	747.3227	1804.413

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145.04	10.13	C5H7NO4	2	2-Oxoglutaramate	8	Amino Acid Metabolism	C00940	19128	0.66	1.00	0.353	1	4028.287	6128.735	1417.412	5780.596
88.016	7.589	C3H4O3	3	Pyruvate	10	Carbohydrate Metabolism	C00022	1066968	0.66	1.00	0.484	1	199529.9	303672.6	67450.66	347307.1
247.14	11.07	C11H21NO5	1	Hydroxybutyrylcarnitine	5	0	0	7078	0.65	1.00	0.308	1	2085.942	3198.058	1624.74	2135.911
152.06	7.84	C7H8N2O2	3	N1-Methyl-2-pyridone-5-carboxamide	7	Metabolism of Cofactors and	C05842	129692	0.65	1.00	0.385	1	24003.93	37065.63	14084.11	41369.5
240.15	11.06	C12H20N2O	2	Slaframine	5	Biosynthesis of Secondary	C06185	3247	0.65	1.00	0.144	1	1065.792	1649.473	402.6322	852.8837
228.21	3.78	C14H28O2	15	Tetradecanoic acid	8	Lipid Metabolism	C06424	329274	0.63	1.00	0.098	1	136618	215624.2	40621.86	95439.35
161.07	9.347	C6H11NO4	10	O-Acetyl-L-homoserine	6	Amino Acid Metabolism	C01077	9683	0.63	1.00	0.238	1	2458.909	3904.988	848.6626	2654.267
329.26	5.152	C18H35NO4	2	4-8dimethylnonanoylcarnitin	7	Lipid Metabolism	0	2984	0.63	1.00	0.002	1	1153.93	1835.94	434.1569	306.7755
186.13	4.013	C10H18O3	19	10-Oxodecanoate	7	Lipids: Fatty Acyls	C02217	22064	0.62	1.00	0.161	1	7095.277	11418.69	3931.637	6395.815
325.23	8.189	C18H31NO4	4	12-Nitro-9Z,12Z-octadecadienoic acid	7	Lipids: Fatty Acyls	C13958	6215	0.62	1.00	0.221	1	1235.434	1998.426	910.6879	1944.276
130.06	4.809	C6H10O3	17	[FA oxo(6:0)] 2-oxo-hexanoic acid	7	Lipids: Fatty Acyls	C00902	1172980	0.62	1.00	0.309	1	251122.6	407700.1	86487.66	356408.6
371.3	4.786	C21H41NO4	1	Tetradecanoylcarnitine	5	0	0	5754	0.62	1.00	0.048	1	2096.756	3408.33	952.6407	1297.961
126.01	11.99	C2H7O4P	2	2-Hydroxyethylphosphonate	6	Amino Acid Metabolism	C06451	10787	0.61	1.00	0.337	1	2856.077	4688.096	1592.402	3503.403
256.09	9.981	C12H16O6	5	Phenylgalactoside	7	0	C02578	26414	0.61	1.00	0.248	1	4855.764	7982.952	5176.98	9515.109
126	10.27	C2H6O4S	1	2-Hydroxyethanesulfonate	6	Amino Acid Metabolism	C05123	16004	0.60	1.00	0.290	1	3037.334	5026.404	1157.453	4871.005
341.26	5.085	C19H35NO4	1	trans-2-Dodecenoylcarnitine	7	0	0	18371	0.60	1.00	0.056	1	5214.154	8709.926	3163.988	4846.862
141.08	8.669	C7H11NO2	3	L-Hypoglycin	7	0	C08287	48843	0.59	1.00	0.342	1	5980.306	10055.29	7088.75	17182.24
343.27	5	C19H37NO4	2	1,2-dioctanoyl-1-amino-2,3-propanediol	5	0	0	13804	0.59	1.00	0.050	1	4452.32	7550.205	2509.83	4207.787

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194.13	4.008	C12H18O2	1	4-Hexyloxyphenol	5	0	C14305	7056	0.59	1.00	0.024	1	2381.983	4041.282	1591.537	2291.941
203.12	10.84	C9H17NO4	1	O-Acetylcarnitine	10	Amino Acid Metabolism	C02571	2303494	0.59	1.00	0.208	1	485619	824403.2	189266.1	672519.5
174	5.32	C6H6O4S	2	Phenol sulfate	7	0	C02180	225171	0.58	1.00	0.126	1	30013.73	51427.29	47494.05	79115.74
182.06	8.705	C9H10O4	13	3-(4-Hydroxyphenyl)lactate	8	Amino Acid Metabolism	C03672	35829	0.58	1.00	0.127	1	9583.478	16480.65	2888.648	9487.206
238.16	3.784	C14H22O3	3	[FA oxo(5:1/5:0/4:0)] (1R,2R)-3-oxo-2-(2'Z-	7	Lipids: Fatty Acyls	0	3284	0.56	1.00	0.147	1	969.2111	1725.698	699.3023	1034.535
142.07	12.26	C6H10N2O2	1	Ectoine	6	Amino Acid Metabolism	C06231	16686	0.55	1.00	0.187	1	2507.62	4529.731	2597.26	5617.223
174.1	11.31	C7H14N2O3	5	N5-Ethyl-L-glutamine	7	0	C01047	61984	0.53	1.00	0.463	1	7577.637	14390.31	12096.3	22356.62
268.24	3.725	C17H32O2	18	omega-Cyclohexylundecanoic acid	5	Lipids: Fatty Acyls	C12100	24002	0.53	1.00	0.068	1	6441.053	12246.64	2394.058	7102.286
138.03	4.774	C7H6O3	7	Gentisate aldehyde	8	Amino Acid Metabolism	C05585	229849	**0.53**	1.00	0.059	1	62178.63	118273.1	35675.89	53910.17
252.21	3.73	C16H28O2	16	[FA (16:2)] 9,12-hexadecadienoic acid	5	Lipids: Fatty Acyls	0	6864	**0.52**	1.00	0.041	1	2242.128	4272.744	615.9503	2092.354
296.27	3.694	C19H36O2	13	[FA methyl(18:0)] 11R,12S-methylene-octadecanoic	5	Lipids: Fatty Acyls	C13838	8146	0.51	1.00	0.072	1	2288.643	4473.286	599.0778	2722.468
285.14	4.396	C17H19NO3	11	N-[(E,E)-Piperoyl]piperidine	7	0	C03882	86557	0.51	1.00	0.101	1	15758.29	31075.63	14192.51	30477.69
90.032	9.336	C3H6O3	6	(R)-Lactate	8	Carbohydrate Metabolism	C00256	18538214	0.51	1.00	0.420	1	2221742	4385598	883798.8	6328268
367.27	4.895	C21H37NO4	1	3, 5-Tetradecadiencarnitine	7	0	0	9451	**0.50**	1.00	0.037	1	2208.313	4420.634	1069.159	2325.002
369.29	4.83	C21H39NO4	2	cis-5-Tetradecenoylcarnitine	7	0	0	22888	0.49	1.00	0.069	1	4843.799	9959.5	2694.101	6344.193
302.22	3.695	C20H30O2	43	[FA (20:5)] 5Z,8Z,11Z,14Z,17Z-	5	Lipids: Fatty Acyls	C06428	16105	0.48	1.00	0.144	1	3188.138	6600.384	1322.161	4768.166
180.08	4.535	C10H12O3	7	Coniferyl alcohol	7	Biosynthesis of Secondary	C00590	102549	0.48	1.00	0.046	1	29151.96	61161.76	22034.05	33902.02
282.26	3.702	C18H34O2	29	[FA (18:1)] 9Z-octadecenoic acid	5	Lipids: Fatty Acyls	C00712	3155771	**0.47**	1.00	0.051	1	833205.6	1778021	208640.2	1009633

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278.22	3.725	C18H30O2	38	[FA (18:3)] 9Z,12Z,15Z-octadecatrienoic acid	5	Lipids: Fatty Acyls	C06427	149734	0.47	1.00	0.037	1	42271	90551.09	22735.16	43087.25
176.03	12.88	C6H8O6	15	Ascorbate	6	Carbohydrate Metabolism	C00072	29462	0.47	1.00	0.430	1	3572.49	7676.91	4137.387	11316.37
226.19	3.801	C14H26O2	12	(9Z)-Tetradecenoic acid	5	Lipids: Fatty Acyls	C08322	78776	0.46	1.00	0.184	1	12857	27673.13	5573.737	26075.17
315.24	5.203	C17H33NO4	2	[FA (10:0)] O-decanoyl-R-carnitine	7	Lipids: Fatty Acyls	C03299	249179	0.46	1.00	0.071	1	42978.07	92897.94	24406.92	78012.85
179.06	7.514	C9H9NO3	6	Hippurate	8	Amino Acid Metabolism	C01586	635412	0.46	1.00	0.138	1	112700.4	244870	80533.8	180763.8
175.06	8.447	C10H9NO2	12	Indole-3-acetate	8	Amino Acid Metabolism	C00954	14327	0.46	1.00	0.092	1	2960.261	6496.5	970.3885	4149.852
198.16	3.866	C12H22O2	17	[PR] Citronellyl acetate	7	Lipids: Prenols	C12298	24862	0.45	1.00	0.217	1	3440.959	7711.777	2243.211	7988.107
289.15	11.1	C13H23NO6	1	3-Methylglutarylcarnitine	7	0	0	12638	0.45	1.00	0.182	1	1718.649	3853.035	1268.402	3967.349
310.29	3.677	C20H38O2	16	[FA (20:0)] 11Z-eicosenoic acid	6	Lipids: Fatty Acyls	C16526	15832	0.44	1.00	0.034	1	4579.143	10457.29	1096.463	4944.698
254.22	3.741	C16H30O2	19	(9Z)-Hexadecenoic acid	6	Lipid Metabolism	C08362	559870	0.42	1.00	0.106	1	96753.05	227712.1	47071.93	189785.8
428.37	3.778	C29H48O2	12	(24R,24'R)-Fucosterol epoxide	7	0	C03910	13685	0.40	1.00	0.035	1	3080.059	7730.257	1200.181	4988.376
149.11	9.671	C6H15NO3	1	Triethanolamine	8	Lipid Metabolism	C06771	55595	0.39	1.00	0.060	1	8384.63	21505.96	4816.508	17342.05
133.05	7.47	C8H7NO	7	Indoxyl	6	Amino Acid Metabolism	C05658	170881	0.36	1.00	0.363	1	11699.9	32302.76	7917.335	61201.69
353.33	3.997	C22H43NO2	1	[FA (20:0)] N-(11Z-eicosaenoyl)-	5	Lipids: Fatty Acyls	0	78325	0.33	1.00	0.148	1	8065.574	24292.14	9575.427	26150.9
351.31	3.979	C22H41NO2	1	[FA (20:2)] N-(11Z,14Z-eicosadienoyl)-	5	Lipids: Fatty Acyls	0	125562	0.33	1.00	0.143	1	12473.31	37965.19	15407.12	42470.83
328.24	3.665	C22H32O2	11	Docosahexaenoicacid	7	Lipids: Fatty Acyls	C06429	109895	0.31	1.00	0.126	1	11054.28	35388.95	3774.486	36936.07
140.06	9.389	C6H8N2O2	7	Methylimidazoleacetic acid	8	Amino Acid Metabolism	C05828	132362	0.30	1.00	0.269	1	7924.341	26436.42	6964.714	46874.3
243.18	3.968	C13H25NO3	1	N-Undecanoylglycine	5	0	0	124422	0.28	1.00	0.091	1	12127.98	43072.43	9329.147	39856.49

Sort	Trend Sort	Import Peaks	Search	Tools	Graphs	Export												
Mass	RT	FORMULA	Isomer	Putative metabolite	confidence	Map	KEGGid#	max intensity	Post	Pre	ttest: Post	ttest: Pre	Mean: Post	Mean: Pre	SD: Post	SD: Pre
182.08	12.86	C6H14O6	6	D-Sorbitol	8	Carbohydrate Metabolism	C00794	111579	0.26	1.00	0.032	1	9643.208	37482.25	10022.53	35026.53
259.14	9.866	C12H21NO5	1	N-(3-oxooctanoyl)-L-homoserine	7	0	0	19993	0.24	1.00	0.354	1	865.4869	3632.507	797.799	7231.336
145.07	11.83	C6H11NO3	9	[FA oxo,amino(6:0)] 3-oxo-5S-amino-hexanoic acid	8	Amino Acid Metabolism	C03656	82015	0.24	1.00	0.161	1	7025.94	29495.21	6963.731	35083.2
132.06	11.33	C3H8N4O2	1	Methylenediurea	5	0	C06381	4527	0.00	1.00	0.243	1	0	233.6403	NA	477.7291
242.09	4.106	C15H14O3	10	Equol	7	0	C14131	80496	0.00	1.00	0.356	NA	0	11499.36	NA	NA

List of References

Abdul-Rahim, A.H., Fulton, R.L., Frank, B., Tatlisumak, T., Paciaroni, M. et al., 2014. Association of improved outcome in acute ischaemic stroke patients with atrial fibrillation who receive early antithrombotic therapy: analysis from VISTA. *European Journal of Neurology,* 0. pp.1-8.

Adams, H.P., Jr., Davis, P.H., Leira, E.C., Chang, K.C., Bendixen, B.H. et al., 1999. Baseline NIH Stroke Scale score strongly predicts outcome after stroke: A report of the Trial of Org 10172 in Acute Stroke Treatment (TOAST). *Neurology,* 53(1). pp.126-131.

Aho, K., Harmsen, P., Hatano, S., Marquardsen, J., Smirnov, V.E. et al., 1980. Cerebrovascular disease in the community: results of a WHO collaborative study. *Bulletin of the World Health Organization,* 58(1). pp.113-130.

Ahrens, W., Pigeot, I. and Wild, P., 2014. Design and planning of epidemiological studies. In: W. Ahrens and I. Pigeot eds. *Handbook of epidemiology*. New York: Springer Science+Business Media. pp. 473-524.

Airee, A., Draper, H.M. and Finks, S.W., 2008. Aspirin resistance: Disparities and clinical implications. *Pharmacotherapy,* 28(8). pp.999-1018.

Airoldi, F., Colombo, A., Morici, N., Latib, A., Cosgrave, J. et al., 2007. Incidence and predictors of drug-eluting stent thrombosis during and after discontinuation of thienopyridine treatment. *Circulation,* 116(7). pp.745-754.

Alberts, M.J., Bergman, D.L., Molner, E., Jovanovic, B.D., Ushiwata, I. et al., 2004. Antiplatelet effect of aspirin in patients with cerebrovascular disease. *Stroke,* 35(1). pp.175-178.

Ali, M., 2008. *The Virtual International Stroke Trials Archive (VISTA): Promulgation of a clinical trial resource*. PhD. University of Glasgow.

Ali, M., Bath, P.M.W., Curram, J., Davis, S.M., Diener, H.-C. et al., 2007. The Virtual International Stroke Trials Archive. *Stroke,* 38(6). pp.1905-1910.

Alli, O., Smith, C., Hoffman, M., Amanullah, S., Katz, P. et al., 2011. Incidence, predictors, and outcomes of gastrointestinal bleeding in patients on dual antiplatelet therapy with aspirin and clopidogrel. *Journal of Clinical Gastroenterology,* 45(5). pp.410-414.

Altman, R., Carreras, L., Diaz, R., Figueroa, E., Paolasso, E. et al., 1994. Collaborative overview of randomized trials of antiplatelet therapy- I: Prevention of death, myocardial-infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ,* 308(6921). pp.81-100.

Amsterdam, E.A., Wenger, N.K., Brindis, R.G., Casey, J.D.E., Ganiats, T.G. et al., 2014. 2014 AHA/ACC guideline for the management of patients with non–st-elevation acute coronary syndromes: A report of the American College of Cardiology/American Heart Association task force on practice guidelines. *Journal of the American College of Cardiology,* 64(24). pp.e139-e228.

Andersen, H.R., Nielsen, T.T., Rasmussen, K., Thuesen, L., Kelbaek, H. et al., 2003a. A comparison of coronary angioplasty with fibrinolytic therapy in acute myocardial infarction. *The New England Journal of Medicine,* 349(8). pp.733-742.

Andersen, H.R., Nielsen, T.T., Vesterlund, T., Grande, P., Abildgaard, U. et al., 2003b. Danish multicenter randomized study on fibrinolytic therapy versus acute coronary angioplasty in acute myocardial infarction: rationale and design of the DANish trial in Acute Myocardial Infarction-2 (DANAMI-2). *American Heart Journal,* 146(2). pp.234-241.

Andersen, K.K., Andersen, Z.J. and Olsen, T.S., 2011. Predictors of early and late case-fatality in a nationwide Danish study of 26,818 patients with first-ever ischemic stroke. *Stroke,* 42(10). pp.2806-2812.

Andrews, R.K. and Berndt, M.C., 2004. Platelet physiology and thrombosis. *Thrombosis Research,* 114(5-6). pp.447-453.

Angiolillo, D.J., 2012. The evolution of antiplatelet therapy in the treatment of acute coronary syndromes: from aspirin to the present day. *Drugs,* 72(16). pp.2087-2116.

Angiolillo, D.J., Fernandez-Ortiz, A., Bernardo, E., Ramirez, C., Sabate, M. et al., 2006. Clopidogrel withdrawal is associated with proinflammatory and prothrombotic effects in patients with diabetes and coronary artery disease. *Diabetes,* 55(3). pp.780-784.

Angiolillo, D.J., Saucedo, J.F., Deraad, R., Frelinger, A.L., Gurbel, P.A. et al., 2010. Increased platelet inhibition after switching from maintenance clopidogrel to prasugrel in patients with acute coronary syndromes: results of the SWAP (SWitching Anti Platelet) study. *Journal of the American College of Cardiology,* 56(13). pp.1017-1023.

Antiplatelet Trialists' Collaboration, 1994. Collaborative overview of randomised trials of antiplatelet therapy - III: Reduction in venous thrombosis and pulmonary embolism by antiplatelet prophylaxis among surgical and medical patients. *BMJ,* 308(6923). pp.235-246.

Arboix, A., Garcia-Eroles, L., Sellares, N., Raga, A., Oliveres, M. et al., 2009. Infarction in the territory of the anterior cerebral artery: clinical study of 51 patients. *BMC Neurology,* 9. pp.30.

Ariesen, M.J., Algra, A., Warlow, C.P. and Rothwell, P.M., 2006. Predictors of risk of intracerebral haemorrhage in patients with a history of TIA or minor ischaemic stroke. *Journal of Neurology, Neurosurgery, and Psychiatry,* 77(1). pp.92-94.

Armstrong, D., Summers, C., Ewart, L., Nylander, S., Sidaway, J.E. et al., 2014. Characterization of the adenosine pharmacology of ticagrelor reveals therapeutically relevant inhibition of equilibrative nucleoside transporter 1. *Journal of Cardiovascular Pharmacology and Therapeutics,* 19(2). pp.209-219.

Armstrong, P.C., Dhanji, A.R., Truss, N.J., Zain, Z.N., Tucker, A.T. et al., 2009. Utility of 96-well plate aggregometry and measurement of thrombi adhesion to determine aspirin and clopidogrel effectiveness. *Thrombosis and Haemostasis,* 102(4). pp.772-778.

Armstrong, P.C., Dhanji, A.R., Tucker, A.T., Mitchell, J.A. and Warner, T.D., 2010. Reduction of platelet thromboxane A2 production ex vivo and in vivo by clopidogrel therapy. *Journal of Thrombosis and Haemostasis,* 8(3). pp.613-615.

Ault, K.A., Cannon, C.P., Mitchell, J., McCahan, J., Tracy, R.P. et al., 1999. Platelet activation in patients after an acute coronary syndrome: results from the TIMI-12 trial. Thrombolysis in Myocardial Infarction. *Journal of the American College of Cardiology,* 33(3). pp.634-639.

B.Dunn, W., Mamas, M. and Heazell, A., 2013. Metabolomics and its role in the study of mammalian systems. In: M. Lämmerhofer and W. Weckwerth eds. *Metabolomics in practice: Successful strategies to generate and analyze metabolic data*. Singapore: Wiley-VCH. pp. 345-377.

Bach, J.P., Riedel, O., Pieper, L., Klotsche, J., Dodel, R. et al., 2011. Health-related quality of life in patients with a history of myocardial infarction and stroke. *Cerebrovascular Diseases,* 31(1). pp.68-76.

Baigent, C., Blackwell, L., Collins, R., Emberson, J., Godwin, J. et al., 2009. Aspirin in the primary and secondary prevention of vascular disease: Collaborative meta-analysis of individual participant data from randomised trials. *Lancet,* 373(9678). pp.1849-1860.

Baigent, C., Sudlow, C., Collins, R. and Peto, R., 2002. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ,* 324(7329). pp.71-86.

Bath, P.M., Lees, K.R., Schellinger, P.D., Altman, H., Bland, M. et al., 2012. Statistical analysis of the primary outcome in acute stroke trials. *Stroke,* 43(4). pp.1171-1178.

Bath, P.M.W., Robson, K., Woodhouse, L.J., Sprigg, N., Dineen, R. et al., 2015. Statistical analysis plan for the 'Triple Antiplatelets for Reducing Dependency

after Ischaemic Stroke' (TARDIS) trial. *International Journal of Stroke,* 10(3). pp.449-451.

Bavry, A.A., Bhatt, D.L. and Topol, E.J., 2007. Experimental antiplatelet therapy. In: A. D. Michelson ed. *Platelets*. United States of America: Elsevier. pp. 1195.

Bavry, A.A., Kumbhani, D.J., Rassi, A.N., Bhatt, D.L. and Askari, A.T., 2006. Benefit of early invasive therapy in acute coronary syndromes: A meta-analysis of contemporary randomized clinical trials. *Journal of the American College of Cardiology,* 48(7). pp.1319-1325.

Bejot, Y., Aboa-Eboule, C., de Maistre, E., Jacquin, A., Troisgros, O. et al., 2013. Prestroke antiplatelet therapy and early prognosis in stroke patients: the Dijon Stroke Registry. *European Journal of Neurology,* 20(6). pp.879-890.

Bell, A.D., Roussin, A., Cartier, R., Chan, W.S., Douketis, J.D. et al., 2011. The use of antiplatelet therapy in the outpatient setting: Canadian Cardiovascular Society guidelines. *Canadian Journal of Cardiology,* 27 Suppl A. pp.S1-59.

Bennett, D., Yan, B., Macgregor, L., Eccleston, D. and Davis, S.M., 2008. A pilot study of resistance to aspirin in stroke patients. *Journal of Clinical Neuroscience,* 15(11). pp.1204-1209.

Berger, J.S., Krantz, M.J., Kittelson, J.M. and Hiatt, W.R., 2009. Aspirin for the prevention of cardiovascular events in patients with peripheral artery disease: a meta-analysis of randomized trials. *JAMA,* 301(18). pp.1909-1919.

Berger, P.B., Bhatt, D.L., Fuster, V., Steg, P.G., Fox, K.A. et al., 2010. Bleeding complications with dual antiplatelet therapy among patients with stable vascular disease or risk factors for vascular disease: results from the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial. *Circulation,* 121(23). pp.2575-2583.

Bertrand, M.E., Legrand, V., Boland, J., Fleck, E., Bonnier, J. et al., 1998. Randomized multicenter comparison of conventional anticoagulation versus antiplatelet therapy in unplanned and elective coronary stenting. The full anticoagulation versus aspirin and ticlopidine (fantastic) study. *Circulation,* 98(16). pp.1597-1603.

Bertrand, M.E., Rupprecht, H.J., Urban, P. and Gershlick, A.H., 2000. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting : the clopidogrel aspirin stent international cooperative study (CLASSICS). *Circulation,* 102(6). pp.624-629.

Bhatt, D.L., Fox, K.A.A., Hacke, W., Berger, P.B., Black, H.R. et al., 2006. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *The New England Journal of Medicine,* 354(16). pp.1706-1717.

Biondi-Zoccai, G.G.L., Lotrionte, M., Agostoni, P., Abbate, A., Fusaro, M. et al., 2006. A systematic review and meta-analysis on the hazards of discontinuing or not adhering to aspirin among 50 279 patients at risk for coronary artery disease. *European Heart Journal,* 27(22). pp.2667-2674.

Boggon, R., van Staa, T.P., Timmis, A., Hemingway, H., Ray, K.K. et al., 2011. Clopidogrel discontinuation after acute coronary syndromes: frequency, predictors and associations with death and myocardial infarction--a hospital registry-primary care linked cohort (MINAP-GPRD). *European Heart Journal,* 32(19). pp.2376-2386.

Boncoraglio, G.B., Bodini, A., Brambilla, C., Corsini, E., Carriero, M.R. et al., 2009. Aspirin resistance determined with PFA-100 does not predict new thrombotic events in patients with stable ischemic cerebrovascular disease. *Clinical Neurology and Neurosurgery,* 111(3). pp.270-273.

Bonello, L., De Labriolle, A., Scheinowitz, M., Lemesle, G., Roy, P. et al., 2009. Emergence of the concept of platelet reactivity monitoring of response to thienopyridines. *Heart,* 95(15). pp.1214-1219.

Bonello, L., Laine, M., Kipson, N., Mancini, J., Helal, O. et al., 2014. Ticagrelor increases adenosine plasma concentration in patients with an acute coronary syndrome. *Journal of the American College of Cardiology,* 63(9). pp.872-877.

Born, G. and Patrono, C., 2006. Antiplatelet drugs. *British Journal of Pharmacology,* 147(S1). pp.S241-S251.

Brainin, M. and Heiss, W.D., eds. 2009. Textbook of stroke medicine. New York, US, Cambridge University Press.

Brass, L.F., 2003. Thrombin and platelet activation*. *Chest,* 124(3_suppl). pp.18S-25S.

Breslow, N.E., 2014. Case-control studies. In: W. Ahrens and I. Pigeot eds. *Handbook of epidemiology*. New York: Springer Science+Business Media. pp. 294-323.

Brewer, D.B., 2006. Max Schultze (1865), G. Bizzozero (1882) and the discovery of the platelet. *British Journal of Haematology,* 133(3). pp.251-258.

Broderick, J.P., Bonomo, J.B., Kissela, B.M., Khoury, J.C., Moomaw, C.J. et al., 2011. Withdrawal of antithrombotic agents and its impact on ischemic stroke occurrence. *Stroke,* 42(9). pp.2509-2514.

Bulluck, H., Kwok, C.S., Ryding, A.D. and Loke, Y.K., 2015. Safety of short-term dual antiplatelet therapy after drug-eluting stents: An updated meta-analysis with direct and adjusted indirect comparison of randomized control trials. *International Journal of Cardiology,* 181. pp.331-339.

Bunag, R.D., Douglas, C.R., Imai, S. and Berne, R.M., 1964. Influence of a pyrimidopyrimidine derivative on deamination of adenosine by blood. *Circulation Research,* 15. pp.83-88.

Burger, W., Chemnitius, J.M., Kneissl, G.D. and Rucker, G., 2005. Low-dose aspirin for secondary cardiovascular prevention - cardiovascular risks after its perioperative withdrawal versus bleeding risks with its continuation - review and meta-analysis. *Journal of Internal Medicine,* 257(5). pp.399-414.

Burn, J., Dennis, M., Bamford, J., Sandercock, P., Wade, D. et al., 1994. Long-term risk of recurrent stroke after a first-ever stroke. The Oxfordshire Community Stroke Project. *Stroke,* 25(2). pp.333-337.

Cannon, C.P., Harrington, R.A., James, S., Ardissino, D., Becker, R.C. et al., 2010. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. *Lancet,* 375(9711). pp.283-293.

Catella-Lawson, F., Reilly, M.P., Kapoor, S.C., Cucchiara, A.J., DeMarco, S. et al., 2001. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *New England Journal of Medicine,* 345(25). pp.1809-1817.

Catella, F., Healy, D., Lawson, J.A. and FitzGerald, G.A., 1986. 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. *Proceedings of the National Academy of Sciences,* 83(16). pp.5861-5865.

Cattaneo, M., Schulz, R. and Nylander, S., 2014. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. *Journal of the American College of Cardiology,* 63(23). pp.2503-2509.

Charlot, M., Nielsen, L.H., Lindhardsen, J., Ahlehoff, O., Olsen, A.M.S. et al., 2012. Clopidogrel discontinuation after myocardial infarction and risk of thrombosis: a nationwide cohort study. *European Heart Journal,* 33(20). pp.2527-2534.

Chen, X.Y., Sun, A.J., Zou, Y.Z., Ge, J.B., Lazar, J.M. et al., 2011. Impact of sphingomyelin levels on coronary heart disease and left ventricular systolic function in humans. *Nutrition and Metabolism,* 8.

Chen, Z.M., Hui, J.M., Liu, L.S., Liu, Z.M., Peto, R. et al., 1997. CAST: Randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. *Lancet,* 349(9066). pp.1641-1649.

Chen, Z.M., Jiang, L.X., Chen, Y.P., Xie, J.X., Pan, H.C. et al., 2005. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet,* 366(9497). pp.1607-1621.

Collet, J.P., Montalescot, G., Blanchet, B., Tanguy, M.L., Golmard, J.L. et al., 2004. Impact of prior use or recent withdrawal of oral antiplatelet agents on acute coronary syndromes. *Circulation,* 110(16). pp.2361-2367.

Costa, F., Vranckx, P., Leonardi, S., Moscarella, E., Ando, G. et al., 2015. Impact of clinical presentation on ischaemic and bleeding outcomes in patients receiving 6-or 24-month duration of dual-antiplatelet therapy after stent implantation: a pre-specified analysis from the PRODIGY(Prolonging Dual-Antiplatelet Treatment After Grading Stent-Induced Intimal Hyperplasia) trial. *European Heart Journal,* 36(20). pp.1242-1251.

Cox, D., Maree, A.O., Dooley, M., Conroy, R., Byrne, M.F. et al., 2006. Effect of enteric coating on antiplatelet activity of low-dose aspirin in healthy volunteers. *Stroke,* 37(8). pp.2153-2158.

D'Ascenzo, F., Colombo, F., Barbero, U., Moretti, C., Omede, P. et al., 2014. Discontinuation of dual antiplatelet therapy over 12 months after acute coronary syndromes increases risk for adverse events in patients treated with percutaneous coronary intervention: systematic review and meta-analysis. *Journal of Interventional Cardiology,* 27(3). pp.233-241.

Das, R., Kilcullen, N., Morrell, C., Robinson, M.B., Barth, J.H. et al., 2006. The British Cardiac Society Working Group definition of myocardial infarction: implications for practice. *Heart,* 92(1). pp.21-26.

Davi, G. and Patrono, C., 2007. Platelet activation and atherothrombosis. *The New England Journal of Medicine,* 357(24). pp.2482-2494.

De Miguel, A., Ibanez, B. and Badimon, J.J., 2008. Clinical implications of clopidogrel resistance. *Thrombosis and Haemostasis,* 100(2). pp.196-203.

Deb, P., Sharma, S. and Hassan, K.M., 2010. Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis. *Pathophysiology,* 17(3). pp.197-218.

Dengler, R., Diener, H.C., Schwartz, A., Grand, M., Schumacher, H. et al., 2010. Early treatment with aspirin plus extended-release dipyridamole for transient ischaemic attack or ischaemic stroke within 24 h of symptom onset (EARLY trial): a randomised, open-label, blinded-endpoint trial. *Lancet Neurology,* 9(2). pp.159-166.

Deo, S.V., Dunlay, S.M., Shah, I.K., Altarabsheh, S.E., Erwin, P.J. et al., 2013. Dual anti-platelet therapy after coronary artery bypass grafting: is there any benefit? A systematic review and meta-analysis. *Journal of Cardiac Surgery,* 28(2). pp.109-116.

Devereaux, P.J., Mrkobrada, M., Sessler, D.I., Leslie, K., Alonso-Coello, P. et al., 2014. Aspirin in patients undergoing noncardiac surgery. *The New England Journal of Medicine,* 370(16). pp.1494-1503.

Dhamoon, M.S., Sciacca, R.R., Rundek, T., Sacco, R.L. and Elkind, M.S.V., 2006. Recurrent stroke and cardiac risks after first ischemic stroke - The Northern Manhattan Study. *Neurology,* 66(5). pp.641-646.

Diehl, P., Halscheid, C., Olivier, C., Helbing, T., Bode, C. et al., 2011. Discontinuation of long term clopidogrel therapy induces platelet rebound hyperaggregability between 2 and 6 weeks post cessation. *Clinical Research in Cardiology,* 100(9). pp.765-771.

Diener, H.C., Bogousslavsky, J. and Brass, L.M., 2004. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-contralled trial. *Lancet,* 364(9446). pp.331-337.

Diener, H.C., Cunha, L., Forbes, C., Sivenius, J., Smets, P. et al., 1996. European Stroke Prevention Study 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. *Journal of the Neurological Sciences,* 143(1). pp.1-13.

Djukanovic, N., Todorovic, Z., Obradovic, S., Zamaklar-Trifunovic, D., Njegomirovic, S. et al., 2011. Abrupt cessation of one-year clopidogrel treatment is not associated with thrombotic events. *Journal of Pharmacological Sciences,* 117(1). pp.12-18.

DÖnges, K., Schiele, R., Gitt, A., Wienbergen, H., Schneider, S. et al., 2001. Incidence, determinants, and clinical course of reinfarction in-hospital after index acute myocardial infarction (results from the pooled data of the maximal individual therapy in acute myocardial infarction [mitra], and the myocardial infarction registry [MIR]). *The American Journal of Cardiology,* 87(9). pp.1039-1044.

Eikelboom, J.W., Hankey, G.J., Thom, J., Bhatt, D.L., Steg, P.G. et al., 2008. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. *Circulation,* 118(17). pp.1705-1712.

Eikelboom, J.W., Hirsh, J., Weitz, J.I., Johnston, M., Yi, Q. et al., 2002. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation,* 105(14). pp.1650-1655.

Elkeles, R.S., Hampton, J.R., Honour, A.J., Mitchell, J.R. and Prichard, J.S., 1968. Effect of a pyrimido-pyrimidine compound on platelet behaviour in vitro and in vivo. *Lancet,* 2(7571). pp.751-754.

Ellero-Simatos, S., Lewis, J.P., Georgiades, A., Yerges-Armstrong, L.M., Beitelshees, A.L. et al., 2014. Pharmacometabolomics reveals that serotonin is implicated in aspirin response variability. *CPT: Pharmacometrics & Systems Pharmacology,* 3(7). pp.e125.

Eltzschig, H.K., 2009. Adenosine: an old drug newly discovered. *Anesthesiology,* 111(4). pp.904-915.

Emmons, P.R., Harrison, M.J., Honour, A.J. and Mitchell, J.R., 1965. Effect of a pyrimidopyrimidine derivative on thrombus formation in the rabbit. *Nature,* 208(5007). pp.255-257.

Englyst, N.A., Horsfield, G., Kwan, J. and Byrne, C.D., 2008. Aspirin resistance is more common in lacunar strokes than embolic strokes and is related to stroke severity. *Journal of Cerebral Blood Flow and Metabolism,* 28(6). pp.1196-1203.

European Medicines Agency, *Prasugrel: Summary of product characteristics.* [online]. Available at:<http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000984/WC500021971.pdf> [Accessed 28 Feb 2016].

Falk, E., Shah, P.K. and Fuster, V., 1995. Coronary plaque disruption. *Circulation,* 92(3). pp.657-671.

Farrell, B., Godwin, J., Richards, S. and Warlow, C., 1991. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. *Journal of Neurology, Neurosurgery and Psychiatry,* 54(12). pp.1044-1054.

Ferrari, E., Benhamou, M., Cerboni, P. and Marcel, B., 2005. Coronary syndromes following aspirin withdrawal - A special risk for late stent thrombosis. *Journal of the American College of Cardiology,* 45(3). pp.456-459.

Ferreira-Gonzalez, I., Marsal, J.R., Ribera, A., Permanyer-Miralda, G., Garcia-Del Blanco, B. et al., 2012. Double antiplatelet therapy after drug-eluting stent implantation risk associated with discontinuation within the first year. *Journal of the American College of Cardiology,* 60(15). pp.1333-1339.

Fleisher, L.A., Fleischmann, K.E., Auerbach, A.D., Barnason, S.A., Beckman, J.A. et al., 2014. 2014 ACC/AHA Guideline on perioperative cardiovascular evaluation and management of patients undergoing noncardiac surgery: Executive summary: A report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. *Circulation,* 130(24). pp.2215-2245.

Fowkes, F.G., Price, J.F., Stewart, M.C., Butcher, I., Leng, G.C. et al., 2010. Aspirin for prevention of cardiovascular events in a general population screened for a low ankle brachial index: a randomized controlled trial. *JAMA,* 303(9). pp.841-848.

Franchi, F., Rollini, F. and Angiolillo, D.J., 2014. Perspectives on the management of antiplatelet therapy in patients with coronary artery disease requiring cardiac and noncardiac surgery. *Current Opinion in Cardiology,* 29(6). pp.553-563.

Frelinger, A.L., 3rd, Furman, M.I., Linden, M.D., Li, Y., Fox, M.L. et al., 2006. Residual arachidonic acid-induced platelet activation via an adenosine diphosphate-dependent but cyclooxygenase-1- and cyclooxygenase-2-independent pathway: a 700-patient study of aspirin resistance. *Circulation,* 113(25). pp.2888-2896.

Fremes, S.E., Levinton, C., Naylor, C.D., Chen, E., Christakis, G.T. et al., 1993. Optimal antithrombotic therapy following aortocoronary bypass: a meta-analysis. *European Journal of Cardio-Thoracic Surgery,* 7(4). pp.169-180.

Frizzell, J.P., 2005. Acute stroke: pathophysiology, diagnosis, and treatment. *AACN Clinical Issues,* 16(4). pp.421-440; quiz 597-428.

Furie, K.L., Kasner, S.E., Adams, R.J., Albers, G.W., Bush, R.L. et al., 2011. Guidelines for the prevention of stroke in patients with stroke or transient ischemic attack a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke,* 42(1). pp.227-276.

Furman, M.I., Benoit, S.E., Barnard, M.R., Valeri, C.R., Borbone, M.L. et al., 1998. Increased platelet reactivity and circulating monocyte-platelet aggregates in patients with stable coronary artery disease. *Journal of the American College of Cardiology,* 31(2). pp.352-358.

Garbe, E. and Suissa, S., 2014. Pharmacoepidemiology. In: W. Ahrens and I. Pigeot eds. *Handbook of epidemiology*. New York: Springer Science+Business Media. pp. 1875-1925.

García Rodríguez, L.A., Cea Soriano, L., Hill, C. and Johansson, S., 2011. Increased risk of stroke after discontinuation of acetylsalicylic acid: A UK primary care study. *Neurology,* 76(8). pp.740-746.

García Rodríguez, L.A. and Hernández-Díaz, S., 2004. Risk of uncomplicated peptic ulcer among users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. *American Journal of Epidemiology,* 159(1). pp.23-31.

GBD 2013 DALYs and HALE Collaborators, 2015. Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition. *The Lancet,* 386(10009). pp.2145-2191.

GBD 2015 Mortality and Causes of Death Collaborators, 2016. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet,* 388(10053). pp.1459-1544.

Geiger, J., Brich, J., Honig-Liedl, P., Eigenthaler, M., Schanzenbacher, P. et al., 1999. Specific impairment of human platelet P2Y(AC) ADP receptor-mediated signaling by the antiplatelet drug clopidogrel. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 19(8). pp.2007-2011.

Gent, M., D Beaumont, J Blanchard, M-G Bousser, J Coffman et al., 1996. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet,* 348. pp.1329-1339.

Gent, M., Easton, J.D., Hachinski, V.C., Panak, E., Sicurella, J. et al., 1989. The Canadian American Ticlopidine Study (CATS) in thromboembolic. *Lancet,* 1. pp.1215-1220.

Ghoshal, K. and Bhattacharyya, M., 2014. Overview of platelet physiology: Its hemostatic and nonhemostatic role in disease pathogenesis. *The Scientific World Journal,* 2014. pp.16.

Giles, M.F. and Rothwell, P.M., 2007. Risk of stroke early after transient ischaemic attack: a systematic review and meta-analysis. *Lancet Neurology,* 6(12). pp.1063-1072.

Giraldo, E.A., 2013. *Ischemic Stroke*. [online]: Merck and the Merck Manuals. Available at:<http://www.merckmanuals.com/professional/neurologic-disorders/stroke-cva/ischemic-stroke> [Accessed 24 Aug 2015].

Gluckman, T.J., McLean, R.C., Schulman, S.P., Kickler, T.S., Shapiro, E.P. et al., 2011. Effects of aspirin responsiveness and platelet reactivity on early vein graft thrombosis after coronary artery bypass graft surgery. *Journal of the American College of Cardiology,* 57(9). pp.1069-1077.

Graipe, A., Binsell-Gerdin, E., Söderström, L. and Mooe, T., 2015. Incidence, time trends, and predictors of intracranial hemorrhage during long-term follow-up after acute myocardial infarction. *J Am Heart Assoc,* 4(12).

Grech, E.D. and Ramsdale, D.R., 2003. Acute coronary syndrome: unstable angina and non-ST segment elevation myocardial infarction. *BMJ (Clinical research ed.),* 326(7401). pp.1259-1261.

Grove, E.L., Hvas, A.M., Mortensen, S.B., Larsen, S.B. and Kristensen, S.D., 2011. Effect of platelet turnover on whole blood platelet aggregation in patients with coronary artery disease. *Journal of Thrombosis and Haemostasis,* 9(1). pp.185-191.

Gupta, S. and Casterella, P.J., 2007. Oral antiplatelet therapy resistance: Definition, diagnosis, and clinical implications. *SIS 2007 Yearbook*. Seattle, WA: Science Innovation Synergy. pp. 1-7.

Gurbel, P.A., Bliden, K.P., Butler, K., Tantry, U.S., Gesheff, T. et al., 2009. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. *Circulation,* 120(25). pp.2577-2585.

Gurbel, P.A. and Tantry, U.S., 2007. Clopidogrel resistance? *Thrombosis Research,* 120(3). pp.311-321.

Guyton, A.C. and Hall, J.E., 2006. *Textbook of medical physiology*. 11th ed. Philadelphia: Elsevier Saunders.

Hacke, W., Kaste, M., Bluhmki, E., Brozman, M., Dávalos, A. et al., 2008a. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *New England Journal of Medicine,* 359(13). pp.1317-1329.

Hacke, W., Ringleb, P.A., Bousser, M.G., Ford, G., Bath, P. et al., 2008b. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008 - The European Stroke Organisation (ESO) Executive Committee and the ESO Writing Committee. *Cerebrovascular Diseases,* 25(5). pp.457-507.

Halkes, P.H.A., A Algra, J van Gijn, Kappelle, L.J. and Koudstaal, P.J., 2006. Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial. *Lancet,* 367. pp.1665-1673.

Hamann, G.F., Weimar, C., Glahn, J., Busse, O. and Diener, H.C., 2003. Adherence to secondary stroke prevention strategies - results from the German Stroke Data Bank. *Cerebrovascular Diseases,* 15(4). pp.282-288.

Hamberg, M., Svensson, J. and Samuelsson, B., 1975. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proceedings of the National Academy of Sciences of the United States of America,* 72(8). pp.2994-2998.

Harker, L.A. and Kadatz, R.A., 1983. Mechanism of action of dipyridamole. *Thrombosis Research. Supplement,* 4. pp.39-46.

Hass, W.K., Easton, J.D., Adams, H.P., Pryse-Phillips, W., Molony, B.A. et al., 1989. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. *The New England Journal of Medicine,* 321(8). pp.501-507.

Held, C., Asenblad, N., Bassand, J.P., Becker, R.C., Cannon, C.P. et al., 2011. Ticagrelor versus clopidogrel in patients with acute coronary syndromes undergoing coronary artery bypass surgery: results from the PLATO (Platelet Inhibition and Patient Outcomes) trial. *Journal of the American College of Cardiology,* 57(6). pp.672-684.

Herlitz, J., Toth, P.P. and Naesdal, J., 2010. Low-dose aspirin therapy for cardiovascular prevention quantification and consequences of poor compliance or discontinuation. *American Journal of Cardiovascular Drugs,* 10(2). pp.125-141.

Hicks, K.A., Tcheng, J.E., Bozkurt, B., Chaitman, B.R., Cutlip, D.E. et al., 2015. 2014 ACC/AHA key data elements and definitions for cardiovascular endpoint events in clinical trials: A report of the American College of Cardiology/American Heart Association task force on clinical data standards (writing committee to develop cardiovascular endpoints data standards). *Journal of the American College of Cardiology,* 66(4). pp.403-469.

Ho, P.M., Magid, D.J., Shetterly, S.M., Olson, K.L., Maddox, T.M. et al., 2008a. Medication nonadherence is associated with a broad range of adverse outcomes in patients with coronary artery disease. *American Heart Journal,* 155(4). pp.772-779.

Ho, P.M., Peterson, E.D., Wang, L., Magid, D.J., Fihn, S.D. et al., 2008b. Incidence of death and acute myocardial infarction associated with stopping clopidogrel after acute coronary syndrome. *JAMA,* 299(5). pp.532-539.

Ho, P.M., Tsai, T.T., Wang, T.Y., Shetterly, S.M., Clarke, C.L. et al., 2010. Adverse events after stopping clopidogrel in post-acute coronary syndrome patients insights from a large integrated healthcare delivery system. *Circulation: Cardiovascular Quality and Outcomes,* 3(3). pp.303-308.

Hoenig, M.R., Aroney, C.N. and Scott, I.A., 2010. Early invasive versus conservative strategies for unstable angina and non-ST elevation myocardial infarction in the stent era. *The Cochrane Database of Systematic Reviews,* (3). pp.Cd004815.

Hong, K.S., Saver, J.L., Kang, D.W., Bae, H.J., Yu, K.H. et al., 2010. Years of optimum health lost due to complications after acute ischemic stroke: disability-adjusted life-years analysis. *Stroke,* 41(8). pp.1758-1765.

Hovens, M.M.C., Snoep, J.D., Eikenboom, J.C.J., van der Bom, J.G., Mertens, B.J.A. et al., 2007. Prevalence of persistent platelet reactivity despite use of aspirin: A systematic review. *American Heart Journal,* 153(2). pp.175-181.

Hubbard, R.B., Smith, C.J., Smeeth, L., Harrison, T.W. and Tattersfield, A.E., 2002. Inhaled corticosteroids and hip fracture: a population-based case-control study. *American Journal of Respiratory and Critical Care Medicine,* 166(12 Pt 1). pp.1563-1566.

Hulot, J.S., Bura, A., Villard, E., Azizi, M., Remones, V. et al., 2006. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood,* 108(7). pp.2244-2247.

Husted, S. and van Giezen, J.J., 2009. Ticagrelor: the first reversibly binding oral P2Y12 receptor antagonist. *Cardiovascular Therapeutics,* 27(4). pp.259-274.

Iakovou, I., Schmidt, T., Bonizzoni, E., Ge, L., Sangiorgi, G.M. et al., 2005. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA,* 293(17). pp.2126-2130.

IBM Corp, 2012. IBM SPSS Statistics for Windows (Version 21.0). Armonk, NY: IBM Corp.

Igarashi, J., Bernier, S.G. and Michel, T., 2001. Sphingosine 1-phosphate and activation of endothelial nitric-oxide synthase. differential regulation of Akt and

MAP kinase pathways by EDG and bradykinin receptors in vascular endothelial cells. *Journal of Biological Chemistry,* 276(15). pp.12420-12426.

Igarashi, J., Erwin, P.A., Dantas, A.P.V., Chen, H. and Michel, T., 2003. VEGF induces S1P(1) receptors in endothelial cells: Implications for cross-talk between sphingolipid and growth factor receptors. *Proceedings of the National Academy of Sciences of the United States of America,* 100(19). pp.10664-10669.

Igarashi, J. and Michel, T., 2001. Sphingosine 1-phosphate and isoform-specific activation of phosphoinositide 3-kinase beta. Evidence for divergence and convergence of receptor-regulated endothelial nitric-oxide synthase signaling pathways. *Journal of Biological Chemistry,* 276(39). pp.36281-36288.

Igarashi, J., Miyoshi, M., Hashimoto, T., Kubota, Y. and Kosaka, H., 2007. Statins induce S1P(1) receptors and enhance endothelial nitric oxide production in response to high-density lipoproteins. *British Journal of Pharmacology,* 150(4). pp.470-479.

International Stroke Trial Collaborative Group, 1997. The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. *Lancet,* 349(9065). pp.1569-1581.

Jankevics, A., Merlo, M.E., de Vries, M., Vonk, R.J., Takano, E. et al., 2012. Separating the wheat from the chaff: a prioritisation pipeline for the analysis of metabolomics datasets. *Metabolomics,* 8(1). pp.S29-S36.

Jauch, E.C., Saver, J.L., Adams, H.P., Bruno, A., Connors, J.J. et al., 2013. Guidelines for the early management of patients with acute ischemic stroke: A guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke,* 44(3). pp.870-947.

Jennings, L.K., 2009. Mechanisms of platelet activation: need for new strategies to protect against platelet-mediated atherothrombosis. *Thrombosis and Haemostasis,* 102(2). pp.248-257.

Jeon, H.W. and Cha, J.K., 2008. Factors related to progression of middle cerebral artery stenosis determined using transcranial Doppler ultrasonograhy. *Journal of Thrombosis and Thrombolysis,* 25(3). pp.265-269.

Jiang, X.C., Paultre, F., Pearson, T.A., Reed, R.G., Francis, C.K. et al., 2000. Plasma sphingomyelin level as a risk factor for coronary artery disease. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 20(12). pp.2614-2618.

Johnson, E.S., Lanes, S.F., Wentworth, C.E., 3rd, Satterfield, M.H., Abebe, B.L. et al., 1999. A metaregression analysis of the dose-response effect of aspirin on stroke. *Archives of Internal Medicine,* 159(11). pp.1248-1253.

Johnston, S.C., Amarenco, P., Albers, G.W., Denison, H., Easton, J.D. et al., 2016. Ticagrelor versus aspirin in acute stroke or transient ischemic attack. *New England Journal of Medicine,* 375(1). pp.35-43.

Kapetanakis, E.I., Medlam, D.A., Petro, K.R., Haile, E., Hill, P.C. et al., 2006. Effect of clopidogrel premedication in off-pump cardiac surgery - Are we forfeiting the benefits of reduced hemorrhagic sequelae? *Circulation,* 113(13). pp.1667-1674.

Karepov, V., Tolpina, G., Kuliczkowski, W. and Serebruany, V., 2008. Plasma triglycerides as predictors of platelet responsiveness to aspirin in patients after first ischemic stroke. *Cerebrovascular Diseases,* 26(3). pp.272-276.

Kastrati, A., Mehilli, J., Schuhlen, H., Dirschinger, J., Dotzer, F. et al., 2004. A clinical trial of abciximab in elective percutaneous coronary intervention after pretreatment with clopidogrel. *The New England Journal of Medicine,* 350(3). pp.232-238.

Kelly, P.J., Crispino, G., Sheehan, O., Kelly, L., Marnane, M. et al., 2012. Incidence, event rates, and early outcome of stroke in Dublin, Ireland: The North Dublin population stroke study. *Stroke,* 43(8). pp.2042-2047.

Kennedy, J., Hill, M.D., Ryckborst, K.J., Eliasziw, M., Demchuk, A.M. et al., 2007. Fast assessment of stroke and transient ischaemic attack to prevent early recurrence (FASTER): a randomised controlled pilot trial. *Lancet Neurology,* 6(11). pp.961-969.

Kernan, W.N., Ovbiagele, B., Black, H.R., Bravata, D.M., Chimowitz, M.I. et al., 2014. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: A guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke,* 45(7). pp.2160-2236.

Kip, K.E., Hollabaugh, K., Marroquin, O.C. and Williams, D.O., 2008. The problem with composite end points in cardiovascular studiesthe story of major adverse cardiac events and percutaneous coronary intervention. *Journal of the American College of Cardiology,* 51(7). pp.701-707.

Koike, H., Asai, F., Sugidachi, A., Kimura, T., Inoue, T. et al., 1993. *Hydropyridine derivatives having antithrombotic activity*. Google Patents. Pat. CA 2077695 A1.

Konig, I.R., Ziegler, A., Bluhmki, E., Hacke, W., Bath, P.M. et al., 2008. Predicting long-term outcome after acute ischemic stroke: a simple index works in patients from controlled clinical trials. *Stroke,* 39(6). pp.1821-1826.

Kour, D., Tandon, V.R., Kapoor, B., Mahajan, A., Parihar, A. et al., 2006. Aspirin resistance. *J K Science,* 8(2). pp.116-117.

Kristofferzon, M.L., Lofmark, R. and Carlsson, M., 2005. Perceived coping, social support, and quality of life 1 month after myocardial infarction: a comparison between Swedish women and men. *Heart and Lung,* 34(1). pp.39-50.

Kulik, A., Chan, V. and Ruel, M., 2009. Antiplatelet therapy and coronary artery bypass graft surgery: perioperative safety and efficacy. *Expert Opin Drug Saf,* 8(2). pp.169-182.

Kulkarni, S.P., Alexander, K.P., Lytle, B., Heiss, G. and Peterson, E.D., 2006. Long-term adherence with cardiovascular drug regimens. *American Heart Journal,* 151(1). pp.185-191.

Kumar, A. and Cannon, C.P., 2009. Acute coronary syndromes: diagnosis and management, part I. *Mayo Clinic Proceedings,* 84(10). pp.917-938.

Kunapuli, S.P., Dorsam, R.T., Kim, S. and Quinton, T.M., 2003. Platelet purinergic receptors. *Current Opinion in Pharmacology,* 3(2). pp.175-180.

Lago, A., Tembl, J.I., Pareja, A., Ponz, A., Ferrer, J.M. et al., 2006. Adherence to aspirin in secondary prevention of ischemic stroke. *Cerebrovascular Diseases,* 21(5-6). pp.353-356.

Lansberg, M.G., O'Donnell, M.J., Khatri, P., Lang, E.S., Nguyen-Huynh, M.N. et al., 2012. Antithrombotic and thrombolytic therapy for ischemic stroke: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest,* 141(2 Suppl). pp.e601S-636S.

Lau, H.S., de Boer, A., Beuning, K.S. and Porsius, A., 1997. Validation of pharmacy records in drug exposure assessment. *Journal of Clinical Epidemiology,* 50(5). pp.619-625.

Lee, M., Wu, Y.-L., Saver, J.L., Lee, H.-C., Lee, J.-D. et al., 2014. Is clopidogrel better than aspirin following breakthrough strokes while on aspirin? A retrospective cohort study. *BMJ Open,* 4(12).

Lee, P.Y., Chen, W.H., Ng, W., Cheng, X., Kwok, J.Y. et al., 2005. Low-dose aspirin increases aspirin resistance in patients with coronary artery disease. *The American Journal of Medicine,* 118(7). pp.723-727.

Lees, K.R., Davalos, A., Davis, S.M., Diener, H.C., Grotta, J. et al., 2006. Additional outcomes and subgroup analyses of NXY-059 for acute ischemic stroke in the SAINT I trial. *Stroke,* 37(12). pp.2970-2978.

Leon , M.B., Baim , D.S., Popma , J.J., Gordon , P.C., Cutlip , D.E. et al., 1998. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. *New England Journal of Medicine,* 339(23). pp.1665-1671.

Lévesque, L.E., Hanley, J.A., Kezouh, A. and Suissa, S., 2010. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. *BMJ,* 340.

Lewis, J.P., Yerges-Armstrong, L.M., Ellero-Simatos, S., Georgiades, A., Kaddurah-Daouk, R. et al., 2013. Integration of pharmacometabolomic and pharmacogenomic approaches reveals novel insights into antiplatelet therapy. *Clinical Pharmacology and Therapeutics,* 94(5). pp.570-573.

Li, J.B., Dong, H.M., Jian, Z., Wu, X.J., Zhao, X.H. et al., 2009. Responsiveness to aspirin in patients with unstable angina pectoris by whole blood aggregometry. *International Journal of Clinical Practice,* 63(3). pp.407-416.

Libby, P. and Theroux, P., 2005. Pathophysiology of coronary artery disease. *Circulation,* 111(25). pp.3481-3488.

Liu, J., Weinhandl, E.D., Gilbertson, D.T., Collins, A.J. and St Peter, W.L., 2012. Issues regarding 'immortal time' in the analysis of the treatment effects in observational studies. *Kidney International,* 81(4). pp.341-350.

Lordkipanidze, M., Diodati, J.G. and Pharand, C., 2009. Possibility of a rebound phenomenon following antiplatelet therapy withdrawal: A look at the clinical and pharmacological evidence. *Pharmacology and Therapeutics,* 123(2). pp.178-186.

Maffrand, J.-P., 2012. The story of clopidogrel and its predecessor, ticlopidine: Could these major antiplatelet and antithrombotic drugs be discovered and developed today? *Comptes Rendus Chimie,* 15(8). pp.737-743.

Maree, A.O., Curtin, R.J., Dooley, M., Conroy, R.M., Crean, P. et al., 2005. Platelet response to low-dose enteric-coated aspirin in patients with stable cardiovascular disease. *Journal of the American College of Cardiology,* 46(7). pp.1258-1263.

Martinez-Pinna, R., Barbas, C., Blanco-Colio, L.M., Tunon, J., Ramos-Mozo, P. et al., 2010. Proteomic and metabolomic profiles in atherothrombotic vascular disease. *Current Atherosclerosis Reports,* 12(3). pp.202-208.

Mason, P.J., Jacobs, A.K. and Freedman, J.E., 2005. Aspirin resistance and atherothrombotic disease. *Journal of the American College of Cardiology,* 46(6). pp.986-993.

Matetzky, S., Shenkman, B., Guetta, V., Shechter, M., Beinart, R. et al., 2004. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation,* 109(25). pp.3171-3175.

Maulaz, A.B., Bezerra, D.C., Michel, P. and Bogousslavsky, J., 2005. Effect of discontinuing aspirin therapy on the risk of brain ischemic stroke. *Archives of Neurology,* 62(8). pp.1217-1220.

Mauri, L., Kereiakes, D.J., Yeh, R.W., Driscoll-Shempp, P., Cutlip, D.E. et al., 2014. Twelve or 30 months of dual antiplatelet therapy after drug-eluting stents. *New England Journal of Medicine,* 371(23). pp.2155-2166.

McConnell, J.P., Cheryk, L.A., Durocher, A., Bruno, A., Bang, N.U. et al., 2001. Urinary 11-dehydro-thromboxane B2 and coagulation activation markers measured within 24 h of human acute ischemic stroke. *Neuroscience Letters,* 313(1–2). pp.88-92.

McGrath, E.R., Kapral, M.K., Fang, J., Eikelboom, J.W., O'Conghaile, A. et al., 2013. Association of atrial fibrillation with mortality and disability after ischemic stroke. *Neurology,* 81(9). pp.825-832.

McTavish, D., Faulds, D. and Goa, K.L., 1990. Ticlopidine. An updated review of its pharmacology and therapeutic use in platelet-dependent disorders. *Drugs,* 40(2). pp.238-259.

Meadows, T.A. and Bhatt, D.L., 2007. Clinical aspects of platelet inhibitors and thrombus formation. *Circulation Research,* 100(9). pp.1261-1275.

Mega, J.L., Close, S.L., Wiviott, S.D., Shen, L., Hockett, R.D. et al., 2009. Cytochrome P-450 polymorphisms and response to clopidogrel. *The New England Journal of Medicine,* 360(4). pp.354-362.

Mehran, R., Baber, U., Steg, P.G., Ariti, C., Weisz, G. et al., 2013. Cessation of dual antiplatelet treatment and cardiac events after percutaneous coronary intervention (PARIS): 2 year results from a prospective observational study. *Lancet,* 382(9906). pp.1714-1722.

Mehta, S.R., Cannon, C.P., Fox, K.A., Wallentin, L., Boden, W.E. et al., 2005. Routine vs selective invasive strategies in patients with acute coronary syndromes: a collaborative meta-analysis of randomized trials. *JAMA,* 293(23). pp.2908-2917.

Mehta, S.R., Tanguay, J.F., Eikelboom, J.W., Jolly, S.S., Joyner, C.D. et al., 2010. Double-dose versus standard-dose clopidogrel and high-dose versus low-dose aspirin in individuals undergoing percutaneous coronary intervention for acute coronary syndromes (CURRENT-OASIS 7): a randomised factorial trial. *Lancet,* 376(9748). pp.1233-1243.

Mehta, S.R., Yusuf, S., Peters, R.J.G., Bertrand, M.E., Lewis, B.S. et al., 2001. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *The Lancet,* 358(9281). pp.527-533.

Michos, E.D., Ardehali, R., Blumenthal, R.S., Lange, R.A. and Ardehali, H., 2006. Aspirin and clopidogrel resistance. *Mayo Clinic Proceedings,* 81(4). pp.518-526.

Miner, J. and Hoffhines, A., 2007. The discovery of aspirin's antithrombotic effects. *Texas Heart Institute Journal,* 34(2). pp.179-186.

Mohan, K.M., Wolfe, C.D.A., Rudd, A.G., Heuschmann, P.U., Kolominsky-Rabas, P.L. et al., 2011. Risk and cumulative risk of stroke recurrence a systematic review and meta-analysis. *Stroke,* 42(5). pp.1489-1494.

Montalescot, G., Bolognese, L., Dudek, D., Goldstein, P., Hamm, C. et al., 2013. Pretreatment with prasugrel in non–ST-segment elevation acute coronary syndromes. *The New England Journal of Medicine,* 369(11). pp.999-1010.

Morisky, D.E., Green, L.W. and Levine, D.M., 1986. Concurrent and predictive-validity of a self-reported measure of medication adherence. *Medical Care,* 24(1). pp.67-74.

Moser, G.H., Schrader, J. and Deussen, A., 1989. Turnover of adenosine in plasma of human and dog blood. *American Journal of Physiology,* 256(4 Pt 1). pp.C799-806.

Motovska, Z., Hlinomaz, O., Miklik, R., Hromadka, M., Varvarovsky, I. et al., 2016. Prasugrel versus ticagrelor in patients with acute myocardial infarction treated with primary percutaneous coronary intervention: Multicenter randomized PRAGUE-18 study. *Circulation*.

Mounib, E.L. and Satchi, T., 2000. *Automating the selection of controls in case-control studies.* [online]. Available at:<http://www2.sas.com/proceedings/sugi25/25/po/25p230.pdf> [Accessed 11 Jan 2015].

Muir, K.W., Weir, C.J., Murray, G.D., Povey, C. and Lees, K.R., 1996. Comparison of neurological scales and scoring systems for acute stroke prognosis. *Stroke,* 27(10). pp.1817-1820.

Muller, I., Besta, F., Schulz, C., Massberg, S., Schonig, A. et al., 2003. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thrombosis and Haemostasis,* 89(5). pp.783-787.

Murray, C.J.L., Richards, M.A., Newton, J.N., Fenton, K.A., Anderson, H.R. et al., 2013. UK health performance: findings of the Global Burden of Disease Study 2010. *The Lancet,* 381(9871). pp.997-1020.

Murray, C.J.L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D. et al., 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet,* 380(9859). pp.2197-2223.

Mylotte, D., Peace, A.J., Tedesco, A.T., Mangiacapra, F., Dicker, P. et al., 2011. Clopidogrel discontinuation and platelet reactivity following coronary stenting. *Journal of Thrombosis and Haemostasis,* 9(1). pp.24-32.

National Institute for Health and Care Excellence, 2010. *Unstable angina and NSTEMI: The early management of unstable angina and non-ST-segment-elevation myocardial infarction (updated Sep 2013)*. (NICE publication no. 94) [online] London: National Institute for Health and Care Excellence. Available at:<http://www.nice.org.uk/guidance/CG94> [Accessed 3 Apr 2015].

National Institute for Health and Care Excellence, 2013. *Myocardial infarction with STsegment elevation: the acute management of myocardial infarction with ST-segment elevation*. (NICE publication no. 167) [online] Manchester: National Institute for Health and Care Excellence. Available at:<http://www.nice.org.uk/guidance/cg167> [Accessed 18 Nov 2013].

National Institute for Health and Clinical Excellence, 2008. *Stroke: Diagnosis and initial management of acute stroke and transient ischaemic attack*. (NICE publication no. 68) [online] London: National Institute for Health and Clinical Excellence. Available at:<https://www.nice.org.uk/guidance/CG68> [Accessed 17 Apr 2014].

National Institute for Health and Clinical Excellence, 2010. *Clopidogrel and modified-release dipyridamole for the prevention of occlusive vascular events*. (NICE publication no. TA210) [online]: National Institute for Health and Clinical Excellence. Available at:<http://www.nice.org.uk/guidance/ta210> [Accessed 8 Sept 2015].

National Institute for Health and Clinical Excellence, 2011. *Ticagrelor for the treatment of acute coronary syndromes*. (NICE publication no. TA236) [online]. Available at:<http://www.nice.org.uk/guidance/ta236> [Accessed 18 Nov 2013].

National Institute for Health and Clinical Excellence, 2012. *Peripheral arterial disease: Diagnosis and management*. (NICE publication no. 147) [online]: National Institute for Health and Clinical Excellence. Available at:<nice.org.uk/guidance/cg147> [Accessed 27 Feb 2016].

National Institute for Health and Clinical Excellence, 2013. *MI – secondary prevention: Secondary prevention in primary and secondary care for patients following a myocardial infarction*. (NICE publication no. 172) [online] London: National Institute for Health and Clinical Excellence. Available at:<http://www.nice.org.uk/guidance/cg172> [Accessed 18 Nov 2013].

National Institute for Health and Clinical Excellence, 2014. *Prasugrel with percutaneous coronary intervention for treating acute coronary syndromes*. (NICE publication no. TA317) [online]: National Institute for Health and Clinical Excellence. Available at:<https://www.nice.org.uk/guidance/ta317> [Accessed 21 Jan 2016].

National Institute of Neurological Disorders and Stroke, 2008. *National Institutes of Health stroke scale*. [online] Bethesda: National Institute of Neurological Disorders and Stroke. Available at:<http://www.ninds.nih.gov/doctors/NIH_Stroke_Scale.pdf> [Accessed 25 Aug 2015].

Nevedomskaya, E., Pacchiarotta, T., Artemov, A., Meissner, A., van Nieuwkoop, C. et al., 2012. (1)H NMR-based metabolic profiling of urinary tract infection: combining multiple statistical models and clinical data. *Metabolomics,* 8(6). pp.1227-1235.

NHS National Services Scotland, *Prescribing Information System (1993 – present).* [online]. Available at:<http://www.isdscotland.org/Health-Topics/Prescribing-and-medicines/Prescribing-Datamarts/docs/PIS_fields_for_researchers_v5_eDRIS%20Guidance.pdf> [Accessed 2 Mar 2016].

NHS National Services Scotland, 2014. *Heart disease statistics.* [online] Scotland: NHS National Services Scotland. Available at:<http://www.isdscotland.org/> [Accessed 18 Aug 2014].

NHS Research Scotland, 2016. *Data Safe Haven.* [online]. Available at:<http://www.nhsresearchscotland.org.uk/research-in-scotland/data/safe-havens> [Accessed 13 July 2016].

O'Brien, J.T., Erkinjuntti, T., Reisberg, B., Roman, G., Sawada, T. et al., 2003. Vascular cognitive impairment. *The Lancet Neurology,* 2(2). pp.89-98.

O'Gara, P.T., Kushner, F.G., Ascheim, D.D., Casey, D.E., Chung, M.K. et al., 2012. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: A report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines. *Circulation.*

Osterberg, L. and Blaschke, T., 2005. Adherence to medication. *The New England Journal of Medicine,* 353(5). pp.487-497.

Overbaugh, K.J., 2009. Acute coronary syndrome. *The American journal of nursing,* 109(5). pp.42-52; quiz 53.

Ozben, S., Ozben, B., Tanrikulu, A.M., Ozer, F. and Ozben, T., 2011. Aspirin resistance in patients with acute ischemic stroke. *Journal of Neurology,* 258(11). pp.1979-1986.

Paciaroni, M., Caso, V. and Agnelli, G., 2009. The concept of ischemic penumbra in acute stroke and therapeutic opportunities. *European Neurology,* 61(6). pp.321-330.

Pang, D., 1999. A relative power table for nested matched case-control studies. *Occupational and Environmental Medicine,* 56(1). pp.67-69.

Park, D.W., Park, S.W., Park, K.H., Lee, B.K., Kim, Y.H. et al., 2006. Frequency of and risk factors for stent thrombosis after drug-eluting stent implantation during long-term follow-up. *The American Journal of Cardiology,* 98(3). pp.352-356.

Park, J. and Gupta, R.S., 2013. Adenosine metabolism, adenosine kinase, and evolution. In: S. Masino and D. Boison eds. *Adenosine: A key link between metabolism and brain activity*. New York: Springer Science+Business Media. pp. 23-54.

Park, Y., Kim, S.B., Wang, B., Blanco, R.A., Le, N.-A. et al., 2009. Individual variation in macronutrient regulation measured by proton magnetic resonance spectroscopy of human plasma. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 297(1). pp.R202-R209.

Patti, G., Colonna, G., Pasceri, V., Pepe, L.L., Montinaro, A. et al., 2005. Randomized trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention: Results from the ARMYDA-2 (antiplatelet therapy for reduction of myocardial damage during angioplasty) study. *Circulation,* 111(16). pp.2099-2106.

Peace, A., McCall, M., Tedesco, T., Kenny, D., Conroy, R.M. et al., 2010. The role of weight and enteric coating on aspirin response in cardiovascular patients. *Journal of Thrombosis and Haemostasis,* 8(10). pp.2323-2325.

Penado, S., Cano, M., Acha, O., Hernandez, J.L. and Riancho, J.A., 2003. Atrial fibrillation as a risk factor for stroke recurrence. *The American Journal of Medicine,* 114(3). pp.206-210.

Pennlert, J., Eriksson, M., Carlberg, B. and Wiklund, P.-G., 2014. Long-term risk and predictors of recurrent stroke beyond the acute phase. *Stroke*.

Petty, G.W., Brown, R.D., Jr., Whisnant, J.P., Sicks, J.D., O'Fallon, W.M. et al., 1999. Ischemic stroke subtypes: a population-based study of incidence and risk factors. *Stroke,* 30(12). pp.2513-2516.

Pulmonary Embolism Prevention (PEP) trial Collaborative Group, 2000. Prevention of pulmonary embolism and deep vein thrombosis with low dose aspirin: Pulmonary Embolism Prevention (PEP) trial. *Lancet,* 355(9212). pp.1295-1302.

Quinn, T.J., Dawson, J., Walters, M.R. and Lees, K.R., 2009. Functional outcome measures in contemporary stroke trials. *International Journal of Stroke,* 4(3). pp.200-205.

Rančić, N., Petrović, B., Apostolović, S., Kocić, B. and Ilić, M., 2013. Health-related quality of life in patients after the acute myocardial infarction. *Central European Journal of Medicine,* 8(2). pp.266-272.

Roe, M.T., Armstrong, P.W., Fox, K.A.A., White, H.D., Prabhakaran, D. et al., 2012. Prasugrel versus clopidogrel for acute coronary syndromes without revascularization. *The New England Journal of Medicine,* 367(14). pp.1297-1309.

Rose, S. and van der Laan, M.J., 2009. Why match? Investigating matched case-control study designs with causal effect estimation. *The International Journal of Biostatistics,* 5(1). pp.1.

Rossini, R., Capodanno, D., Lettieri, C., Musumeci, G., Nijaradze, T. et al., 2011. Prevalence, predictors, and long-term prognosis of premature discontinuation of oral antiplatelet therapy after drug eluting stent implantation. *The American Journal of Cardiology,* 107(2). pp.186-194.

Roth, G.J. and Majerus, P.W., 1975. The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. *The Journal of Clinical Investigation,* 56(3). pp.624-632.

Rothwell, P.M., Algra, A., Chen, Z., Diener, H.C., Norrving, B. et al., 2016. Effects of aspirin on risk and severity of early recurrent stroke after transient ischaemic attack and ischaemic stroke: time-course analysis of randomised trials. *Lancet,* 388(10042). pp.365-375.

Rothwell, P.M., Coull, A.J., Giles, M.F., Howard, S.C., Silver, L.E. et al., 2004. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). *Lancet,* 363(9425). pp.1925-1933.

Rothwell, P.M., Giles, M.F., Chandratheva, A., Marquardt, L., Geraghty, O. et al., 2007. Effect of urgent treatment of transient ischaemic attack and minor stroke on early recurrent stroke (EXPRESS study): a prospective population-based sequential comparison. *Lancet,* 370(9596). pp.1432-1442.

Sabatine, M.S., Cannon, C.P., Gibson, C. and et al., 2005a. Effect of clopidogrel pretreatment before percutaneous coronary intervention in patients with st-elevation myocardial infarction treated with fibrinolytics: The PCI-CLARITY study. *JAMA,* 294(10). pp.1224-1232.

Sabatine, M.S., Cannon, C.P., Gibson, C.M., Lopez-Sendon, J.L., Montalescot, G. et al., 2005b. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *The New England Journal of Medicine,* 352(12). pp.1179-1189.

Sacco, R.L., Diener, H., Yusuf, S., Cotton, D., Ounpuu, S. et al., 2008. Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. *The New England Journal of Medicine,* 359(12). pp.1238-1251.

Sacco, R.L., Kasner, S.E., Broderick, J.P., Caplan, L.R., Connors, J.J. et al., 2013. An updated definition of stroke for the 21st century: A statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke,* 44(7). pp.2064-2089.

Sachdeva, A., Bavisetty, S., Beckham, G., Shen, A.Y.J., Aharonian, V. et al., 2012. Discontinuation of long-term clopidogrel therapy is associated with death and

myocardial infarction after saphenous vein graft percutaneous coronary intervention. *Journal of the American College of Cardiology,* 60(23). pp.2357-2363.

Saka, O., McGuire, A. and Wolfe, C., 2009. Cost of stroke in the United Kingdom. *Age and Ageing,* 38(1). pp.27-32.

Sambu, N., Dent, H., Englyst, N., Warner, T.D., Leadbeater, P. et al., 2011a. Effect of clopidogrel withdrawal on platelet reactivity and vascular inflammatory biomarkers 1 year after drug-eluting stent implantation: results of the prospective, single-centre CESSATION study. *Heart,* 97(20). pp.1661-1667.

Sambu, N., Warner, T. and Curzen, N., 2011b. Clopidogrel withdrawal: Is there a "rebound" phenomenon? *Thrombosis and Haemostasis,* 105(2). pp.211-220.

Sandercock, P.A., Counsell, C., Tseng, M.C. and Cecconi, E., 2014. Oral antiplatelet therapy for acute ischaemic stroke. *The Cochrane Database of Systematic Reviews,* 3. pp.CD000029.

Sandercock, P.A.G., Counsell, C., Gubitz, G.J. and Tseng, M.C., 2008. Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database of Systematic Reviews,* (3).

Saposnik, G., Kapral, M.K., Liu, Y., Hall, R., O'Donnell, M. et al., 2011. IScore: a risk score to predict death early after hospitalization for an acute ischemic stroke. *Circulation,* 123(7). pp.739-749.

Saver, J.L., 2007. Novel end point analytic techniques and interpreting shifts across the entire range of outcome scales in acute stroke trials. *Stroke,* 38(11). pp.3055-3062.

Savi, P. and Herbert, J.M., 2005. Clopidogrel and ticlopidine: P2Y12 adenosine diphosphate-receptor antagonists for the prevention of atherothrombosis. *Seminars in Thrombosis and Hemostasis,* 31(2). pp.174-183.

Scalone, G., Coviello, I., Barone, L., Battipaglia, I., Aurigemma, C. et al., 2011. Evidence of increased platelet reactivity in the first six months after acute ST segment elevation myocardial infarction. *Thrombosis Research,* 128(2). pp.174-178.

Schlitt, A., Blankenberg, S., Yan, D., von Gizycki, H., Buerke, M. et al., 2006. Further evaluation of plasma sphingomyelin levels as a risk factor for coronary artery disease. *Nutrition and Metabolism,* 3(1). pp.1-8.

Schomig, A., Neumann, F.J., Kastrati, A., Schuhlen, H., Blasini, R. et al., 1996. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. *The New England Journal of Medicine,* 334(17). pp.1084-1089.

Schultheiss, H.P., Tschoepe, D., Esser, J., Schwippert, B., Roesen, P. et al., 1994. Large platelets continue to circulate in an activated state after myocardial infarction. *European Journal of Clinical Investigation,* 24(4). pp.243-247.

Schwartz, K.A., Schwartz, D.E., Barber, K., Reeves, M. and De Franco, A.C., 2008. Non-compliance is the predominant cause of aspirin resistance in chronic coronary arterial disease patients. *Journal of Translational Medicine,* 6. pp.46.

Scottish Intercollegiate Guidelines Network (SIGN), 2008. *Management of patients with stroke or TIA: assessment, investigation, immediate management and secondary prevention.* (SIGN publication no. 108) [online] Edinburgh: SIGN. Available at:<http://www.sign.ac.uk/pdf/sign108.pdf> [Accessed 18 Dec 2013].

Scottish Intercollegiate Guidelines Network (SIGN), 2013a. *Acute coronary syndromes.* (SIGN publication no. 93) [online] Edinburgh: SIGN. Available at:<http://www.sign.ac.uk/> [Accessed 18 Nov 2013].

Scottish Intercollegiate Guidelines Network (SIGN), 2013b. *Antithrombotics: indications and management.* (SIGN publication no. 129) [online] Edinburgh: SIGN. Available at:<http://www.sign.ac.uk/guidelines/fulltext/129/> [Accessed 1 Nov 2013].

Serebruany, V., Cherala, G., Williams, C., Surigin, S., Booze, C. et al., 2009. Association of platelet responsiveness with clopidogrel metabolism: role of compliance in the assessment of "resistance". *American Heart Journal,* 158(6). pp.925-932.

Serebruany, V.L., Steinhubl, S.R., Berger, P.B., Malinin, A.I., Baggish, J.S. et al., 2005a. Analysis of risk of bleeding complications after different doses of aspirin in 192,036 patients enrolled in 31 randomized controlled trials. *The American Journal of Cardiology,* 95(10). pp.1218-1222.

Serebruany, V.L., Steinhubl, S.R., Berger, P.B., Malinin, A.I., Bhatt, D.L. et al., 2005b. Variability in platelet responsiveness to clopidogrel among 544 individuals. *Journal of the American College of Cardiology,* 45(2). pp.246-251.

Shalansky, S.J., Levy, A.R. and Ignaszewski, A.P., 2004. Self-reported Morisky score for identifying nonadherence with cardiovascular medications. *Annals of Pharmacotherapy,* 38(9). pp.1363-1368.

Shankar, H., Garcia, A., Prabhakar, J., Kim, S. and Kunapuli, S.P., 2006. P2Y(12) receptor-mediated potentiation of thrombin-induced thromboxane A(2) generation in platelets occurs through regulation of Erk1/2 activation. *Journal of Thrombosis and Haemostasis,* 4(3). pp.638-647.

Shireman, T.I., Howard, P.A., Kresowik, T.F. and Ellerbeck, E.F., 2004. Combined anticoagulant-antiplatelet use and major bleeding events in elderly atrial fibrillation patients. *Stroke,* 35(10). pp.2362-2367.

Sibbing, D., Morath, T., Stegherr, J., Braun, S., Vogt, W. et al., 2009. Impact of proton pump inhibitors on the antiplatelet effects of clopidogrel. *Thrombosis and Haemostasis,* 101(4). pp.714-719.

Sibbing, D., Stegherr, J., Braun, S., Mehilli, J., Schulz, S. et al., 2010. A double-blind, randomized study on prevention and existence of a rebound phenomenon of platelets after cessation of clopidogrel treatment. *Journal of the American College of Cardiology,* 55(6). pp.558-565.

Smith, C.A., Want, E.J., O'Maille, G., Abagyan, R. and Siuzdak, G., 2006. XCMS: Processing mass spectrometry data for metabolite profiling using Nonlinear peak alignment, matching, and identification. *Analytical Chemistry,* 78(3). pp.779-787.

Smith, J.B. and Willis, A.L., 1971. Aspirin selectively inhibits prostaglandin production in human platelets. *Nature: New Biology,* 231(25). pp.235-237.

Smith, P.K., Goodnough, L.T., Levy, J.H., Poston, R.S., Short, M.A. et al., 2012. Mortality benefit with prasugrel in the TRITON-TIMI 38 coronary artery bypass grafting cohort: risk-adjusted retrospective data analysis. *Journal of the American College of Cardiology,* 60(5). pp.388-396.

Smolina, K., Wright, F.L., Rayner, M. and Goldacre, M.J., 2012. Long-term survival and recurrence after acute myocardial infarction in England, 2004 to 2010. *Circulation: Cardiovascular Quality and Outcomes,* 5(4). pp.532-540.

Sofi, F., Giusti, B., Marcucci, R., Gori, A.M., Abbate, R. et al., 2011. Cytochrome P450 2C19*2 polymorphism and cardiovascular recurrences in patients taking clopidogrel: a meta-analysis. *The pharmacogenomics journal,* 11(3). pp.199-206.

Stegemann, C., Pechlaner, R., Willeit, P., Langley, S.R., Mangino, M. et al., 2014. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation,* 129(18). pp.1821-1831.

Steinhubl, S.R., Berger, P.B., Mann, J.T., 3rd, Fry, E.T., DeLago, A. et al., 2002. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA,* 288(19). pp.2411-2420.

Stephenson, J.J., Chang, C.L., Wygant, G.D., Hauch, O. and Cziraky, M.J., 2011. Incidence of death and recurring acute coronary syndrome after stopping clopidogrel therapy in a large commercially-insured population in the US. *Current Medical Research and Opinion,* 27(6). pp.1079-1087.

Storey, R.F., 2011. Pharmacology and clinical trials of reversibly-binding P2Y12 inhibitors. *Thrombosis and Haemostasis,* 105(99). pp.S75-S81.

Storey, R.F., Bliden, K.P., Patil, S.B., Karunakaran, A., Ecob, R. et al., 2010. Incidence of dyspnea and assessment of cardiac and pulmonary function in patients with stable coronary artery disease receiving ticagrelor, clopidogrel, or

placebo in the ONSET/OFFSET study. *Journal of the American College of Cardiology,* 56(3). pp.185-193.

Sud, A., Kline-Rogers, E.M., Eagle, K.A., Fang, J.M., Armstrong, D.F. et al., 2005. Adherence to medications by patients after acute coronary syndromes. *Annals of Pharmacotherapy,* 39(11). pp.1792-1797.

Sugidachi, A., Asai, F., Ogawa, T., Inoue, T. and Koike, H., 2000. The in vivo pharmacological profile of CS-747, a novel antiplatelet agent with platelet ADP receptor antagonist properties. *British Journal of Pharmacology,* 129(7). pp.1439-1446.

Sugidachi, A., Asai, F., Yoneda, K., Iwamura, R., Ogawa, T. et al., 2001. Antiplatelet action of R-99224, an active metabolite of a novel thienopyridine-type G(i)-linked P2T antagonist, CS-747. *British Journal of Pharmacology,* 132(1). pp.47-54.

Tanguay, J.-F., Bell, A.D., Ackman, M.L., Bauer, R.D.C., Cartier, R. et al., 2013. Focused 2012 update of the canadian cardiovascular society guidelines for the use of antiplatelet therapy. *Canadian Journal of Cardiology,* 29(11). pp.1334-1345.

Tantry, U.S., Bliden, K.P., Wei, C., Storey, R.F., Armstrong, M. et al., 2010. First analysis of the relation between CYP2C19 genotype and pharmacodynamics in patients treated with ticagrelor versus clopidogrel: the ONSET/OFFSET and RESPOND genotype studies. *Circulation: Cardiovascular genetics,* 3(6). pp.556-566.

The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995. Tissue plasminogen activator for acute ischemic stroke. *New England Journal of Medicine,* 333(24). pp.1581-1587.

The SALT Collaborative Group, 1991. Swedish Aspirin Low-Dose Trial (SALT) of 75 mg aspirin as secondary prophylaxis after cerebrovascular ischaemic events. *Lancet,* 338(8779). pp.1345-1349.

The SPS3 Investigators, 2012. Effects of clopidogrel added to aspirin in patients with recent lacunar stroke. *New England Journal of Medicine,* 367(9). pp.817-825.

Thon, J.N. and Italiano, J.E., 2010. Platelet formation. *Seminars in Hematology,* 47(3). pp.220-226.

Thune, J.J., Signorovitch, J.E., Kober, L., McMurray, J.J., Swedberg, K. et al., 2011. Predictors and prognostic impact of recurrent myocardial infarction in patients with left ventricular dysfunction, heart failure, or both following a first myocardial infarction. *European Journal of Heart Failure,* 13(2). pp.148-153.

Thygesen, K., Alpert, J.S., Jaffe, A.S., Simoons, M.L., Chaitman, B.R. et al., 2012. Third universal definition of myocardial infarction. *Circulation,* 126(16). pp.2020-2035.

Valgimigli, M., Campo, G., Monti, M., Vranckx, P., Percoco, G. et al., 2012. Short- versus long-term duration of dual-antiplatelet therapy after coronary stenting: a randomized multicenter trial. *Circulation,* 125(16). pp.2015-2026.

van Giezen, J.J. and Humphries, R.G., 2005. Preclinical and clinical studies with selective reversible direct P2Y12 antagonists. *Seminars in Thrombosis and Hemostasis,* 31(2). pp.195-204.

van Giezen, J.J., Sidaway, J., Glaves, P., Kirk, I. and Bjorkman, J.A., 2012. Ticagrelor inhibits adenosine uptake in vitro and enhances adenosine-mediated hyperemia responses in a canine model. *Journal of Cardiovascular Pharmacology and Therapeutics,* 17(2). pp.164-172.

Varenhorst, C., Jensevik, K., Jernberg, T., Sundström, A., Hasvold, P. et al., 2014. Duration of dual antiplatelet treatment with clopidogrel and aspirin in patients with acute coronary syndrome. *European Heart Journal,* 35(15). pp.969-978.

Varga-Szabo, D., Pleines, I. and Nieswandt, B., 2008. Cell adhesion mechanisms in platelets. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 28(3). pp.403-412.

Wahlgren, N., Ahmed, N., Davalos, A., Hacke, W., Millan, M. et al., 2008. Thrombolysis with alteplase 3-4.5 h after acute ischaemic stroke (SITS-ISTR): an observational study. *Lancet,* 372(9646). pp.1303-1309.

Wallentin, L., Becker, R.C., Budaj, A., Cannon, C.P., Emanuelsson, H. et al., 2009a. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *The New England Journal of Medicine,* 361(11). pp.1045-1057.

Wallentin, L., Becker, R.C., Budaj, A., Cannon, C.P., Emanuelsson, H. et al., 2009b. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *New England Journal of Medicine,* 361(11). pp.1045-1057.

Wang, L., Wang, X. and Chen, F., 2010. Clopidogrel resistance is associated with long-term thrombotic events in patients implanted with drug-eluting stents. *Drugs R D,* 10(4). pp.219-224.

Wang, Y., Wang, Y., Zhao, X., Liu, L., Wang, D. et al., 2013. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *The New England Journal of Medicine,* 369(1). pp.11-19.

Wasserman, J.K., Perry, J.J., Sivilotti, M.L.A., Sutherland, J., Worster, A. et al., 2014. Computed tomography identifies patients at high risk for stroke after transient ischemic attack/nondisabling stroke: Prospective, multicenter cohort study. *Stroke,* 46. pp.114-119.

Weiss, H.J., Aledort, L.M. and Kochwa, S., 1968. The effect of salicylates on the hemostatic properties of platelets in man. *Journal of Clinical Investigation,* 47(9). pp.2169-2180.

Wiviott, S.D., Braunwald, E., McCabe, C.H., Montalescot, G., Ruzyllo, W. et al., 2007. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *The New England Journal of Medicine,* 357(20). pp.2001-2015.

World Health Organization, 2004a. *Deaths from coronary heart disease*. [online] Brighton: Myriad Editions Limited. Available at:<http://www.who.int/cardiovascular_diseases/en/cvd_atlas_14_deathHD.pdf > [Accessed 27 Aug 2014].

World Health Organization, 2004b. *Deaths from stroke*. [online] Brighton: Myriad Editions Limited. Available at:<http://www.who.int/cardiovascular_diseases/en/cvd_atlas_16_death_from_stroke.pdf> [Accessed 27 Aug 2014].

World Health Organization, 2004c. *Global burden of coronary heart disease*. [online] Brighton: Myriad Editions Limited. Available at:<http://www.who.int/cardiovascular_diseases/en/cvd_atlas_13_coronaryHD.pdf> [Accessed 28 Aug 2014].

World Health Organization, 2004d. *Global burden of stroke*. [online] Brighton: Myriad Editions Limited. Available at:<http://www.who.int/cardiovascular_diseases/en/cvd_atlas_15_burden_stroke.pdf> [Accessed 27 Aug 2014].

World Health Organization (WHO) Collaborating Centre, 2003. *Introduction to drug utilization research*. [online] Norway: WHO. Available at:<http://www.whocc.no/filearchive/publications/drug_utilization_research.pdf> [Accessed 30 May 2016].

Wykrzykowska, J.J., Warnholtz, A., de Jaeger, P., Curzen, N., Oldroyd, K.G. et al., 2009. Effect of clopidogrel discontinuation at 1 year after drug eluting stent placement on soluble CD40L, P-selectin and C-reactive protein levels: DECADES (Discontinuation Effect of Clopidogrel After Drug Eluting Stent): a multicenter, open-label study. *Journal of Thrombosis and Thrombolysis,* 28(4). pp.410-417.

Yang, W., Chen, Y., Xi, C., Zhang, R., Song, Y. et al., 2013. Liquid chromatography-tandem mass spectrometry-based plasma metabonomics delineate the effect of metabolites' stability on reliability of potential biomarkers. *Analytical Chemistry,* 85(5). pp.2606-2610.

Yerges-Armstrong, L.M., Ellero-Simatos, S., Georgiades, A., Zhu, H., Lewis, J.P. et al., 2013. Purine pathway implicated in mechanism of resistance to aspirin therapy: pharmacometabolomics-informed pharmacogenomics. *Clinical Pharmacology and Therapeutics,* 94(4). pp.525-532.

Yin, P., Lehmann, R. and Xu, G., 2015. Effects of pre-analytical processes on blood samples used in metabolomics studies. *Analytical and Bioanalytical Chemistry,* 407(17). pp.4879-4892.

Yusuf, S., Fox, K.A.A., Tognoni, G., Mehta, S.R., Chrolavicius, S. et al., 2001. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *The New England Journal of Medicine,* 345(7). pp.494-502.

Zimmermann, N. and Hohlfeld, T., 2008. Clinical implications of aspirin resistance. *Thrombosis and Haemostasis,* 100(3). pp.379-390.

Zinkstok, S.M. and Roos, Y.B., 2012. Early administration of aspirin in patients treated with alteplase for acute ischaemic stroke: a randomised controlled trial. *Lancet,* 380(9843). pp.731-737.